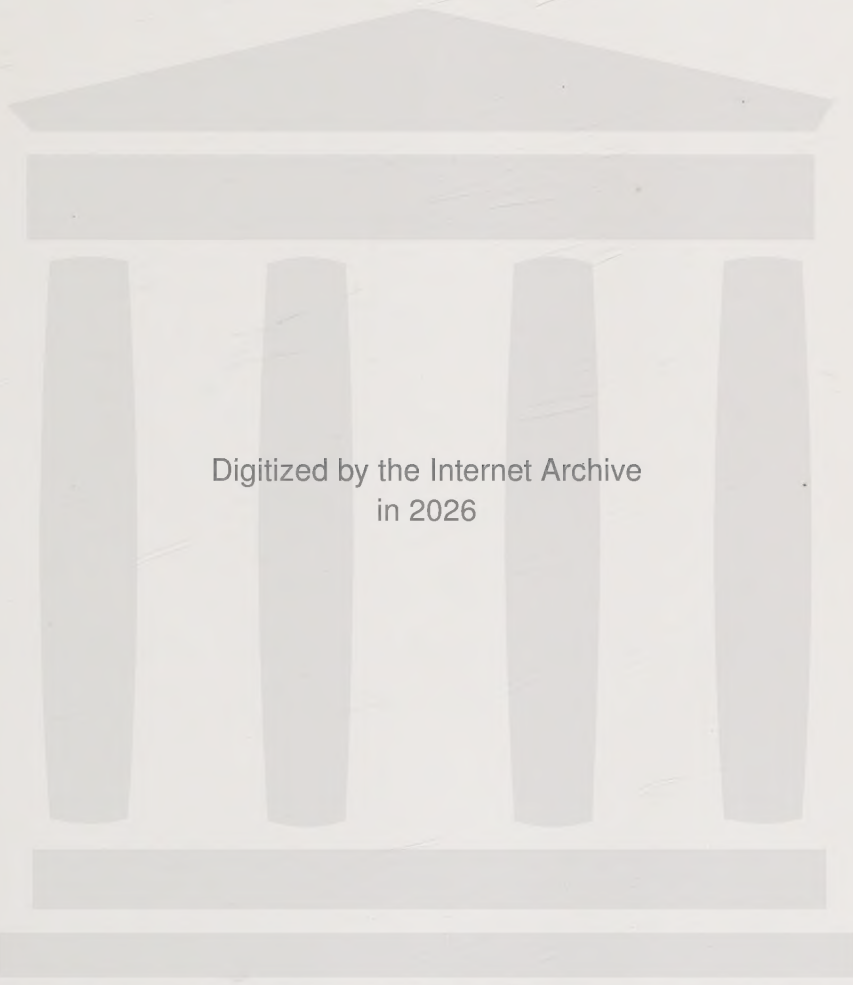


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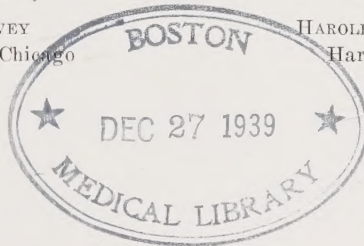
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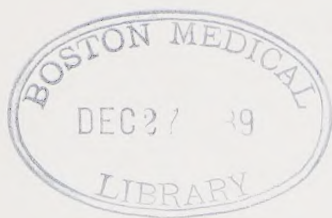


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PROLONGED VAGINAL BLEEDING AND FETAL RESORPTION IN THE ALBANY STRAIN OF RATS ¹

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THREE TEXT FIGURES AND ONE PLATE (SIX FIGURES)

In a previous report Bryan, Klinck and Wolfe ('38) described a strain of rats, referred to as the Albany or A-S strain, in which, coincident with a frequent occurrence of spontaneous mammary fibroadenomata in the females, there was a progressive decline in fertility. In spite of the low fertility attempts are being made, by the method of in-breeding and selection, toward developing lines of higher tumor incidence. During the course of these experiments several factors have become apparent as causes of the low fertility in this colony. Appreciable numbers of the males, for example, become sterile because of a spontaneous degeneration of the germinal epithelium. Large numbers of the females, as previously noted (Bryan, Klinck and Wolfe, '38), exhibit atypical estrous cycles and fail to mate. Finally, a certain percentage of the females that do mate exhibit prolonged vaginal bleeding and resorption of some or all of the fetuses. It is the purpose of this communication to discuss this last group of abnormalities which are associated with the pregnant state.

¹ These investigations were carried out with the aid of a grant from the International Cancer Research Foundation.

An abstract of this paper was given before the American Association of Anatomists, Boston, April, 1939.

MATERIALS AND METHODS

In this study we have analyzed 400 pregnancies occurring among 174 breeding females of the Albany strain. Vaginal smears were made daily on all rats from the time of mating. Inasmuch as the earliest sign of successful implantation of the fertilized ova is manifested in the rat by the appearance of blood in the vagina (known as the 'placental sign') which usually persists from the fourteenth to the seventeenth days (Long and Evans, '21), data were collected concerning the time of the first appearance of macroscopic vaginal bleeding and the number of days during which bleeding occurred. The first day of pregnancy was taken as the day following that on which spermatozoa were noted in the vaginal smear. If that evidence was lacking, the last cornified smear, when accurate identification could be made of the 'heat' period, was taken as the day of insemination.

From the first day of vaginal bleeding the uterine contents were palpated daily in order to follow the course of fetal development. Usually the fetuses could be detected as distinct nodular masses at about the thirteenth day, and the progressive increase or diminution in their size could then be followed. Furthermore, in many cases variations in the consistency of the fetal masses could be detected. Thus by this method the presence of resorbing sites could usually be determined. Frequent examination of the animals was made during the last days of pregnancy in order to ascertain as accurately as possible the length of the gestation period. If delivery occurred during the working day, a record was made of the number of dead and viable young which were born. The animals were not observed between 5.00 P.M. and 8.00 A.M.

In order to obtain more accurate information concerning the nature of the resorbing process complete necropsies were performed on thirty-five animals at various stages of late pregnancy. The whole reproductive tract was removed and fixed in Bouin's fluid. Specimens were dehydrated and cleared in cedar oil, at which stage they were photographed. Such specimens gave considerable information concerning the con-

dition of the contents of the gestational sacs. The fetal sites were then separated and embedded. Fifteen sites were sectioned serially and studied histologically.

Control observations were made on a totally different strain of rats which originated from a colony developed at the Vanderbilt University School of Medicine. A total of 140 pregnancies were analyzed. Both groups of rats were fed a commercially prepared food mixture (Wayne Dog Food

TABLE 1 A COMPARISON of the ALBANY and VANDERBILT STRAINS of RATS BASED on the NUMBER of ANIMALS SHOWING RESORPTION and the NUMBER of PREGNANCIES RE- SULTING in COMPLETE RESORPTION						
	ALBANY STRAIN		VANDERBILT STRAIN			
	TOTAL NO.	PER CENT	NORMAL BREEDING		FORCED BREEDING	
			TOTAL NO.	PER CENT	TOTAL NO.	PER CENT
BREEDING FEMALES	174		37		30	
FEMALES WITH HISTORY OF RESORBING	44	25	1	2.7	0	—
PREGNANCIES	400		140		135	
COMPLETE RESORPTIONS	64	16	1	0.07	0	—

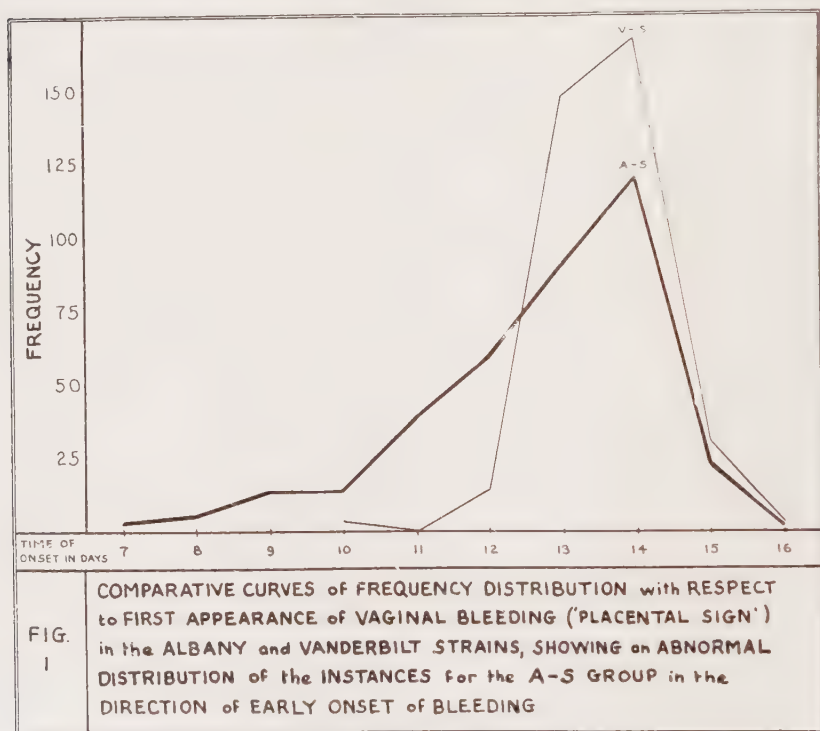
Blox²). This diet was supplemented with lettuce once or twice a week. On this diet the growth and reproductive performance of the rats from the Vanderbilt Colony is excellent.

RESULTS

This study establishes that one of the factors contributing to the low fertility in the Albany strain of rats is the occurrence of spontaneous fetal resorption. Table 1 summarizes the findings. Of 174 breeding females, forty-four animals (25% of the breeders) manifested complete resorption of fetuses at least once during the period under investigation. Sixty-

² Manufactured by the Allied Mills, Inc., Chicago, Ill.

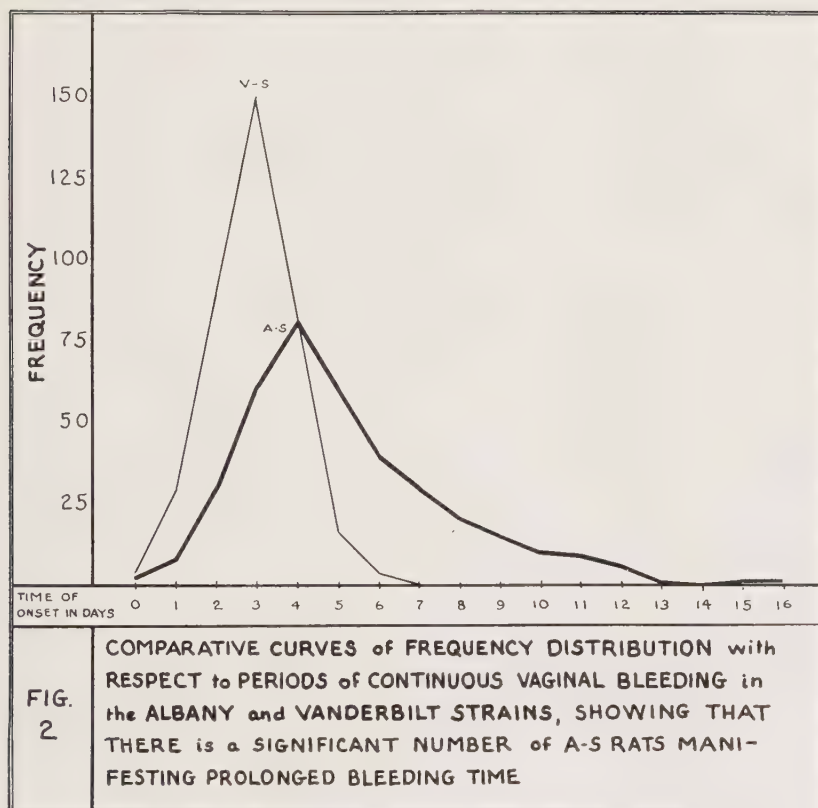
four pregnancies (or 16% of the 400 analyzed) resulted in complete fetal resorption. In striking contrast, animals of the Vanderbilt strain showed complete resorption only once in 140 pregnancies. More recently, in another group of Vanderbilt rats which were forced to breed continuously without the intervention of a lactation phase, for the purpose



of another experiment, the records show that not one resorption occurred in 135 pregnancies.

It was early noted that many pregnant animals of the Albany strain showed a premature appearance of vaginal bleeding referred to as the 'placental sign,' and, furthermore, that this bleeding was much more profuse than that which occurred in the rats of the Vanderbilt colony. When one examines comparative curves of frequency distribution with respect to the

time of onset of this sign in the Albany and Vanderbilt strains (fig. 1), one notices at once that there is an abnormal distribution of observations for the Albany group in the direction of early onset. In figure 2, it will be seen that in a significant number of the A-S breeding females the bleeding



time was prolonged. Both A-S curves are skewed and extended over a wider range of units than the curves of the Vanderbilt series; nevertheless it is clear that the animals of the Albany strain tend not only to show vaginal bleeding earlier but also to bleed over a longer period than do the control females.

In table 2 there is presented a statistical analysis of the observations already discussed and of the average number of young per pregnancy. The differences between the two strains are self evident. The probability that random sampling was responsible for these results was tested by estimating in each case the ratio of the difference between the means to the probable error of this difference. The ratios are

TABLE 2 A COMPARISON of the ALBANY and VANDERBILT STRAINS of RATS BASED on (1) FIRST APPEARANCE of VAGINAL BLEEDING (2) DURATION of BLEEDING and (3) SIZE of LITTER PER PREGNANCY					
OBSERVATION	ALBANY STRAIN		VANDERBILT STRAIN		DIFFERENCE BETWEEN MEANS *
	400 PREGNANCIES		140 PREGNANCIES		
	MEAN $\pm$ P.E. _m	STANDARD DEVIATION $\pm$ P.E. _{σ}	MEAN $\pm$ P.E. _m	STANDARD DEVIATION $\pm$ P.E. _{σ}	P.E. of DIFF.
FIRST APPEARANCE of VAGINAL BLEEDING	12.7 $\pm$ 0.06	1.6 $\pm$ 0.04	13.6 $\pm$ 0.06	0.83 $\pm$ 0.04	11.4
TOTAL DAYS of BLEEDING	5.2 $\pm$ 0.09	2.6 $\pm$ 0.07	2.9 $\pm$ 0.07	1.07 $\pm$ 0.05	20.1
NUMBER of YOUNG PER PREGNANCY	5.4 $\pm$ 0.12	3.5 $\pm$ 0.09	8.7 $\pm$ 0.15	2.5 $\pm$ 0.11	17.0
* FORMULA: $\frac{M_1 - M_2}{\sqrt{(P.E.m_1)^2 + (P.E.m_2)^2}}$ WHEN THIS RATIO IS 3 OR OVER, IT IS CONSIDERED STATISTICALLY SIGNIFICANT					

given in the last column and all signify these differences to be so large as to supersede any likelihood that they are the result of fortuitous sampling.

The number of fetuses which were either born dead, or which died after they were dropped but before they were found by the examiner, represented 6% of the total young delivered in the Albany group and 3% in the Vanderbilt strain. The difference between the two colonies in this respect is not marked and both figures are within the range noted in another

laboratory as occurring sporadically in a large breeding colony.³

The question arose as to whether the size of the litter delivered could be correlated with the number of days during which the animals manifested vaginal bleeding. A prolonged interval of bleeding was associated, to a greater or less degree, with a lower litter yield. The data were highly variable and gave a correlation coefficient of 0.57 ± 0.03 . Hence, so far as prediction is concerned, the chances of predicting the size of the litter from the number of days of bleeding are not significantly enhanced (only about 10%), but the coefficient does indicate that there are common factors operating in both variables that were measured. One of the common factors is probably a primary damage of the maternal and fetal placenta, the histopathology of which will be described presently.

When pregnancy resulted in complete resorption, there was great variability with respect to the onset of this process. Animals which were destined to resorb early in pregnancy with few exceptions showed vaginal bleeding between the ninth and eleventh days (occasionally on the twelfth or thirteenth day). If resorption occurred relatively late in pregnancy (e.g., from the seventeenth day on), the 'placental sign' was not always premature in its appearance but was noted as a rule between the eleventh to fourteenth days. Invariably, however, bleeding was continuous until resorption was complete, at least in so far as could be judged by palpation of the abdomen.

Nearly a third of the gestations which terminated without delivery extended beyond the twenty-third day (24 to 28 days). In these cases the fetuses were fairly well developed at the time of fetal death, and resorption, when it occurred, was necessarily prolonged. In many instances the animals died while attempting to deliver the dead fetuses or suc-

³ Personal communication from Mr. Robert Reinhart of the Yale Laboratory of Physiological Chemistry.

cumbed presumably from the intoxication set up by the necrotic uterine contents.

Observations on the length of the period of gestation which terminated by the delivery of young gave a mean value for the Albany strain of 22.5 days and for the control animals of 21.8 days. In their studies on the length of gestation in the rat, Long and Evans ('21) obtained a mean of 21.8 days involving a probable error of not more than 8 hours. D'Amour and Dumont ('37), who made hourly examination during the day and bi-hourly at night, obtained a value of 21.9 days as the mean of eighty-six control observations. These agreements with our findings for the control group indicate that our observations were fairly accurate and that the inefficiency of the coarse grouping was offset by the large number of observations for the Albany colony.

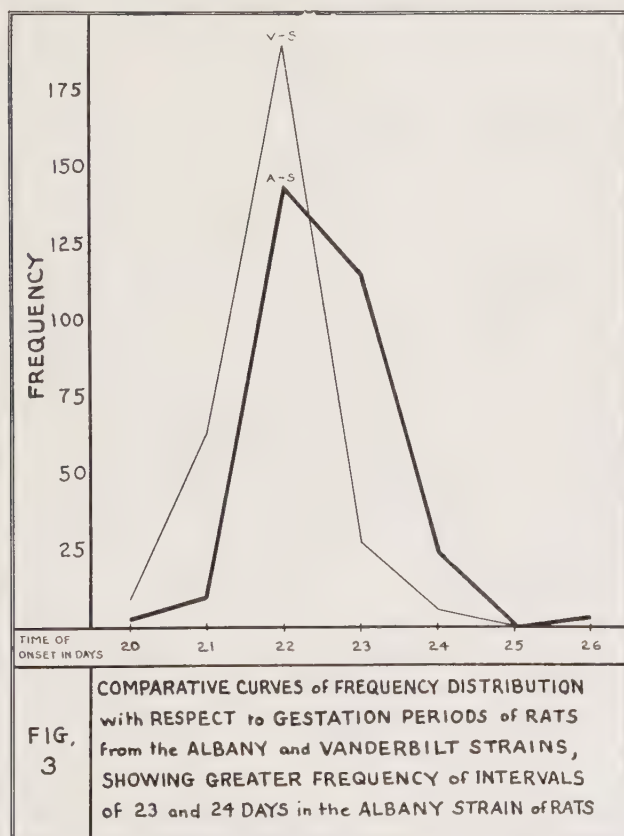
The frequency distribution of gestation periods for both series is given in figure 3. The difference between the means is not significant, and 23 days must be considered within the normal range. However, it is noteworthy that only about 2% of the animals in the control group delivered as late as the twenty-fourth day, whereas 8% of the deliveries among the A-S animals occurred on the twenty-fourth day. Two A-S rats littered as late as the twenty-sixth day, but in both cases the young were born dead. When delivery was made on the twenty-fourth day, in about half of the litters all the young were born alive; in about one-fourth all were born dead; while in the remaining fourth the litters which were dropped consisted of both dead and viable young. Hence, in approximately half of the cases the prolongation of gestation may have been due to the presence of dead fetuses in utero (Mason, '35).

Finally, there was a group of animals which delivered young but retained one or more fetuses. The number of young actually delivered was taken as the number for that litter, and the gestation was considered to be terminated when the incomplete delivery was made. The fetuses which were re-

tained were subsequently either resorbed or became degenerated.

Gross appearance of the pregnant uteri of rats exhibiting spontaneous fetal resorptions

It was found that by the method of palpation the presence of resorbing fetal sites could be detected with a considerable



degree of accuracy. To check the method, however, the pregnant uteri of thirty-five rats between the tenth and nineteenth days of pregnancy were actually examined. The findings compared favorably with the observations made by palpation.

Study of this material indicated that there was a marked variation in the number of fetal sites undergoing resorption. In only a few uteri was resorption occurring at all sites. The resorbing sites were variable in size and appearance, indicating that the beginning time and rate of resorption were not uniform (figs. 4, 5 and 6).

The resorbing sites were generally characterized by the presence of varying degrees of hemorrhage which apparently originated at the lateral margin of the placenta and passed into the uterine cavity and sometimes into the non-pregnant portions of the uterus between the gestational sacs. The resorbing sites were purplish-red in color. In some instances they were somewhat swollen; in others they were shrunken. The impression was gained that early in the resorbing process the fetal sites tended to be swollen but that in later stages they assumed a shrunken condition. This shrinkage was probably the result of dehydration. In many instances the whole site was so hemorrhagic that no details could be made out in the cleared specimens (fig. 6). On the other hand, it was found that a considerable degree of hemorrhage could exist in the fetal enlargement and the fetus appear grossly normal. In their gross appearance the pregnant uteri of these animals resembled those described by Mason ('35) in rats fed an A-deficient diet and by Maeder ('37) in rats subsisting on a fat-deficient diet.

It should be pointed out that in the control pregnancies a small amount of hemorrhage often occurred at the lateral margin of the placenta; that free blood was sometimes found in the uterus between the fetal sites, and that one or two very small resorbing sites were not infrequently seen (fig. 4). On the basis of these observations, it is felt that, when resorption occurs in these animals, as it apparently does, the process must start very early in pregnancy.

Some animals in which resorption sites were palpated late in pregnancy failed to deliver. A few of them were killed, usually after the twenty-fifth day of pregnancy. In some instances the uteri of these contained resorption sites only

and in others fairly large and partly degenerated dead fetuses, but in most of the animals both dead fetuses and resorption sites were found.

Histologic appearance of resorbing fetal sites. The histologic appearance of the resorbing fetal sites varied depending on the degree of resorption and the time at which the resorption began. As far as we have been able to discern, the earliest change occurred in the lateral margin of the placenta at the junction of the decidua basalis and the decidua reflexa. Here, distention of the maternal sinuses occurred together with hemorrhage into the decidual tissue and the adjoining trophoblastic epithelium (see fig. 9 and compare with fig. 7). In the more advanced resorptions necrosis of both the decidua basalis and decidua reflexa occurred. The latter layer was often completely destroyed and allowed the hemorrhage to escape into the adjoining uterine lumen (fig. 8), sometimes causing distention of the site. From this point it seemed that the pathologic process became more marked. The whole of the decidua basalis and decidua reflexa was destroyed; the latter was replaced by a necrotic and hemorrhagic mass. Focal areas of necrosis and hemorrhage were found in the adjoining fetal trophoblast. Reichert's membrane and the vitelline membrane often became distorted and in advanced resorptions were almost destroyed (fig. 9). Although infection was not a predominating characteristic of the resorbing sites in these animals, the necrosis in both the decidua and the trophoblast was often associated with a marked acute inflammatory reaction. The fetal tissue underwent destruction in the resorbing sites (fig. 9), but the changes in the fetus seemed to be secondary to the placental lesion and probably resulted from a lack of nutrient material. In the most advanced resorptions all that remained in the fetal site were the remnants of the decidua basalis which presented many areas of necrosis. Adjoining this were the remains of the trophoblastic epithelium usually more or less widely separated by hemorrhage. The decidua reflexa over the lining epithelium of the uterus which usually passes over it was replaced by

a structureless mass containing cellular debris. Remnants of fetal tissue and of the fetal membranes were seen lying in the yolk sac; varying amounts of blood were found free in the uterine cavity and to a lesser extent in the yolk sac (figs. 8 and 9).

DISCUSSION

A survey of the literature indicates that the course of pregnancy can be affected by a wide variety of experimental procedures involving limitations in diet or disturbances of endocrine balance. It is a matter of some interest that a fairly high percentage of the females in the Albany strain of rats exhibit a spontaneous disturbance of the pregnant state which is similar in some respects to the conditions induced by experimental procedures, and we believe it to be of importance to ascertain the conditions which lead to this poor reproductive performance.

Mason ('35), studying rats which were subsisting on diets deficient in vitamin A, noted premature and extensive vaginal bleeding, fetal resorption, and prolonged and difficult parturition (see also Tansley, '36 and Newton, '38). Maeder ('37) found the same abnormalities in rats which were fed diets free of fat. The histopathological changes in the placenta as described by these investigators are similar to those which occurred spontaneously in appreciable numbers of animals of the Albany strain, although we did not observe as much evidence of infection as was reported by Mason. Spontaneous resorption such as has been observed in our animals differs from resorption which results from vitamin E deficiency in that the process in the latter condition begins by rarefaction of the mesodermal elements of the fetus before any direct involvement of the placenta occurs (Evans, Burr and Althausen, '27, and Uner, '31), while in the rats of our colony the primary lesion is in the placenta, fetal degeneration and resorption occurring later. Resorption of fetuses has been reported by Guilbert and Goss ('32) as occurring in rats subjected to a dietary regimen low in protein, and disturbances

in reproduction have been observed in rats which were fed diets deficient in sodium (Miller, '26, and Orent-Keiles, Robinson and McCollum, '37).

Inasmuch as the ration which was offered the animals of the Albany strain was complete, in so far as could be judged by the excellent growth and reproduction that were observed in the control Vanderbilt colony which was subjected to the same dietary regimen, manifestations of dietary deficiency would not be expected unless one or both of the following conditions prevailed, namely 1) impairment of the mechanism of absorption in these rats, or 2) an increase in their requirement for certain foodstuffs. The possibility that these factors are operating cannot be excluded. The animals apparently were not suffering from vitamin A deficiency, for vaginal cornification failed to persist after castration; as Mason and Wolfe ('35) have shown, the persistence of cornified cells in the castrate vaginal smear serves as an early sign of the lack of this vitamin.

Interference with the maintenance of pregnancy and with the normal processes of parturition has been described in certain instances of experimental endocrine imbalance. We have no evidence that the disturbances noted in the Albany rats can be due to hyperthyroidism (Weichert, '30) or hyperestrinism (Hain, '35; Parkes, Dodds and Noble, '38). The findings reported by Pencharz and Long ('33) and by Selye, Collip and Thomson ('33) on the effect of hypophysectomy upon pregnancy are, however, of pertinent interest. Pencharz and Long observed that hypophysectomy in pregnant rats after implantation of the ovum but before the tenth day resulted in marked vaginal hemorrhage and resorption of the fetuses. On the other hand, hypophysectomy after the tenth day resulted not only in a continuation but also in a prolongation of pregnancy. Essentially similar results were reported by Selye and his collaborators, except that they found that hypophysectomy between the tenth and fourteenth days might result in fetal resorption rather than prolongation of pregnancy. The observations of these investigators concerning the

effect of hypophysectomy on the pregnant state are quite similar to observations made by us on many of pregnant animals of the Albany strain, and they suggest the possibility that certain rats in this strain exhibit some inherent deficiency in either the production or release of anterior lobe hormones. This possibility is strengthened by the fact that many rats show atypical vaginal cycles, and in certain males spontaneous degeneration of the germinal epithelium of the testes has been noted.

SUMMARY

Analysis was made of the course of gestation in a colony of rats, namely, the Albany strain, in which the incidence of spontaneous mammary fibroadenomata is high and the fertility is low. The results were compared statistically with control observations made on a strain in which the reproductive performance is excellent. Significant differences were obtained indicating that the rats of the Albany strain 1) show vaginal bleeding or the 'placental sign' earlier in pregnancy 2) bleed more profusely and over a longer period of time and 3) yield a lower average of young per litter than do the control animals. The smaller average number of young delivered is the result of the high frequency of spontaneous fetal resorptions.

A study of the gross appearance of the uteri of the rats exhibiting spontaneous fetal resorption revealed a marked variation in the number of resorbing sites, the time of onset of the resorbing process, and the rate of resorption. Grossly, the resorbing foci were characterized by hemorrhage into the fetal sites which were swollen in some instances and shrunken in others. Histologically, the resorbing sites were hemorrhagic, and there was marked tissue destruction in the lateral margins of the placenta. Destruction of fetal tissue was secondary to the placental lesions.

Observations were made of the length of the gestation period in both series. It was noted that although most of the deliveries in the Albany strain were made within 21 to 23 days, 8% occurred as late as the twenty-fourth day. In general,

pregnancies among the animals of the Albany strain took one of the following courses: 1) uneventful gestation, 2) resorption of some or all of the fetuses, 3) retention of fetuses beyond the normal period of pregnancy; these were either resorbed or became degenerated and necrotic, 4) delivery of some of the fetuses and retention and subsequent resorption or degeneration of the others. The last two courses were not infrequently terminated by the death of the females.

The possible relationship of dietary or endocrinological factors to these abnormalities is discussed.

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PLATE 1

EXPLANATION OF FIGURES

4 Uterus taken from a Vanderbilt control rat on the fourteenth day of pregnancy. Notice one small resorption site and hemorrhage between some of the gestational sacs.

5 Uterus taken from a rat of the Albany strain on the fourteenth day of pregnancy. Note both normal and resorbing sites and the irregularity in the progress of the resorbing process.

6 Uterus taken from a rat of the Albany strain on the seventeenth day of pregnancy. All fetal sites are undergoing resorption.

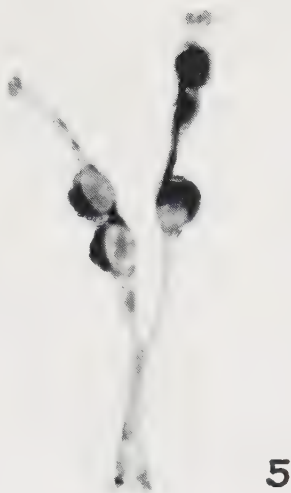
7 Section through gestational sac of a normal Vanderbilt rat killed on the fourteenth day of pregnancy. $\times 10$.

8 Section through gestational sac of an Albany rat killed on the eighteenth day of pregnancy. Note marked hemorrhage and degenerate appearance of maternal and fetal placenta. $\times 10$.

9 Section through gestational sac of an Albany rat killed on the fourteenth day of pregnancy. Note hemorrhage at the lateral margin of placenta and degenerate remains of fetus. $\times 10$.



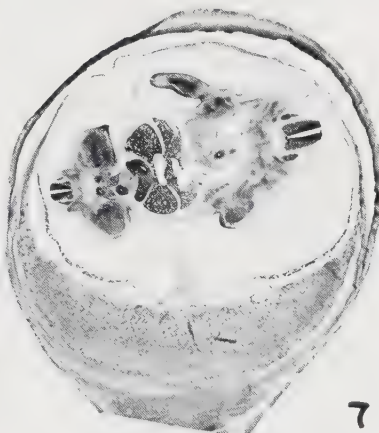
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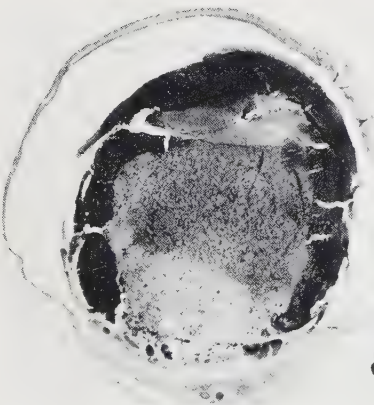
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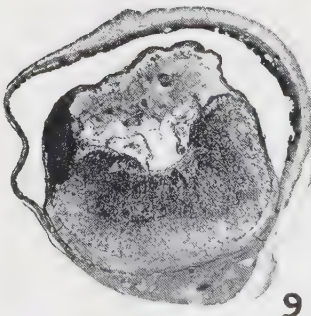
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PARTHENOGENIC CLEAVAGE IN THE HUMAN OVARY

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ONE FIGURE

Pathologists and gynecologists have long been interested in parthenogenesis as supplying a possible mechanism for the explanation of the occurrence of teratoma in the ovary and testis (Waldeyer, 1872). The following evidence of parthenogenesis in man is presented although it is not the author's concept of the etiology of teratoma (Krafka, '38).

In many forms of life, it is of course well known that an egg may undergo development without the stimulus of the sperm. In other forms where fertilization is the normal course of events, the egg may be stimulated to initiate development by the simple expedient of shaking, puncturing or changing the density or temperature of the surrounding medium.

In mammals, maturation of the ovum proceeds while the egg is still within the unruptured follicle. In the event that ovulation does not occur, the ovum may continue to develop through the early cleavage stages. This phenomenon is so common among such mammals as the rat, mouse and guinea pig that at present we use sections of ovary for study of maturation in our course in embryology. Polar bodies and reduction spindles are easily found.

In many of these slides atretic follicles are present which show definite cleavage stages including the two cell stage, four cell, eight cell and some few more advanced stages. That these are cleavage stages rather than the fusion of two or more eggs in polyovular follicles (an interpretation made by

Kampmeier, '29) is clear since an occasional cleavage spindle is seen. We have however never seen a stage advanced as far as an embryonic vesicle such as described by Loeb and made the factual background of his teratoma theory (Loeb, '12).

The purpose of this paper is to put on record observations bearing on the process of parthenogenesis in man. To our

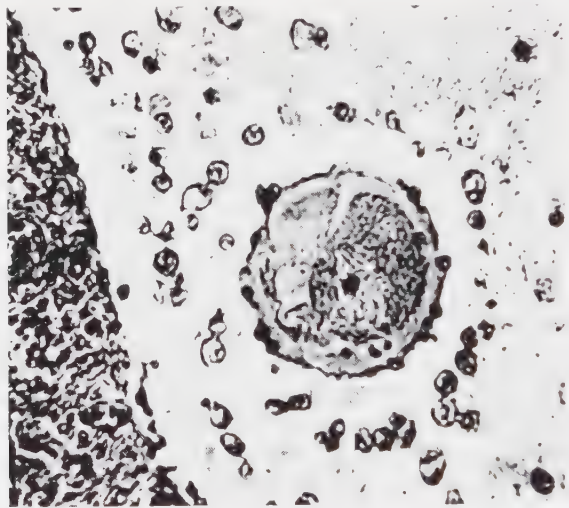


Fig.1 Untouched photomicrograph of an ovum undergoing cleavage in an atretic follicle in the ovary of a 7 year-old child. One large discrete blastomere is set off sharply from a second large cell and two smaller cells. These four cells all lie within a discrete slightly thickened membrane, the zona pellucida. No invasive granulosa cells are seen. Note the disruption of the stratum granulosum and the corona radiata. Magnification $\times 100$.

knowledge this phenomenon has been recorded only once, namely by Häggström ('22).

Our sections are from the ovary of a 7-year-old child. The tissue shows typical ovarian structure including many follicles undergoing atresia in stages varying from granulosa disintegration to advanced corpora albicantes spuriae. One follicle contains an egg that is definitely made up of four blastomeres. These lie within a definite thickened membrane,

the zona pellucida, hence they cannot possibly be interpreted as fusion products of a polyovular follicle (fig. 1). No invasive granulosa cells are within the membrane, as frequently occurs in some ova hence this point of common confusion is not involved.

The only additional concept that might be advanced is that of fragmentation of a degenerating egg since the nuclei are not conspicuous. This point is admitted and it might leave the question of parthenogenesis in the higher mammals open, were it not for the beautiful illustration of a similar cleavage stage in an atretic follicle presented by photomicrograph by Saglik ('38) for the gibbon.

From the foregoing findings we predict that a careful routine examination by pathologists of all eggs in atretic follicles will uncover abundant material supporting the viewpoint of parthenogenic cleavage in man.

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THE RELATION OF THE MEDIAN NERVE TO THE PRONATOR TERES MUSCLE ¹

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ONE FIGURE

Variations in the relation of the median nerve to the pronator teres muscle are not described or figured in the anatomical texts commonly used in this country. It is the authors' purpose to call attention to these variations, and to furnish a brief account of the frequency of their occurrence. The study is based upon the examination of 240 consecutive arms of American whites and negroes, comprising 120 right and 120 left arms.

The usual relationship, which is the textbook's 'normal,' is one in which the median nerve passes between the ulnar and humeral heads of the pronator teres muscle. This arrangement was encountered in 198 of the 240 arms in our series, that is, in 82.5% of the cases (fig. 1a). Three other types of relation between the nerve and the muscle were observed: first, that in which the ulnar head was lacking,² the median nerve passing beneath the humeral head (fig. 1b; 21 cases or 8.75%); second, an arrangement in which the nerve passed behind the ulnar head (fig. 1c; 15 cases or

¹Contribution no. 289 from the Anatomical Laboratories of Northwestern University Medical School.

²The frequent absence of the ulnar head of the pronator teres muscle has been remarked by many anatomists, among them Macalister (1868), Wood (1868), LeDouble (1897), Chudzinski (1898) and Bryce ('23). Macalister suggested that the ulnar head arose from an accessory 'embryonic germ,' thus accounting for the frequency of its deficiency. According to Kolster ('01), Bryce, Wood, and others, the ulnar head is marked in monotremes, but is absent in mammals generally, including the lower primates; among the primates, it occurs in the orang and chimpanzee, but not in the gibbon or gorilla.

6.25%); third, one in which the nerve pierced the humeral head, finding passage between groups of the component fascicles (fig. 1d; 6 cases or 2.5%). Differences in the percentage occurrence of the four varieties in right and left arms were discovered, but cannot be regarded as significant, since the number of specimens examined was small (table 1).

The only readily available record on the percentage occurrence of these varieties is that of Lanz and Wachsmuth ('35); these writers found that the nerve passed between the two heads of the muscle in 95.5% of specimens (authors' 82.5%), behind the ulnar head in 2% (authors' 6.25%), through the

TABLE 1
Relation of median nerve to pronator teres muscle

<i>Relation</i>	<i>Fig. no.</i>	<i>% occurrence all arms</i>	<i>% occurrence right arms</i>	<i>% occurrence left arms</i>
Nerve between heads of muscle	1a	82.5 (198 of 240)	84.1 (101 of 120)	80.8 (97 of 120)
Nerve under humeral head (ulnar missing)	1b	8.75 (21 of 240)	7.5 (9 of 120)	10.0 (12 of 120)
Nerve under ulnar head	1c	6.25 (15 of 240)	6.67 (8 of 120)	5.83 (7 of 120)
Nerve through humeral head	1d	2.5 (6 of 240)	1.67 (2 of 120)	3.34 (4 of 120)

humeral head in 1.5% (authors' 2.5%), and behind the humeral head alone when the ulnar is absent in 1% (authors' 8.75%).

In connection with anomalies of the pronator teres muscle, other courses of the median nerve past the site of the muscle have been reported, none of which, however, have been seen by the present authors. The nerve may be overlapped by an accessory head arising in the upper arm: such cases have been recorded by Bryce ('23), Grosjurin ('31), Hallett (1849)

Fig. 1 Four types of interrelationship of the medial nerve and the pronator teres muscle, arranged in the order of their frequency. Volar surface of the arm and forearm dissected to the muscular level; the brachioradialis and flexor carpi radialis muscles have been retracted to the lateral and the medial sides, respectively in order to expose the pronator teres muscle and the median nerve. The percentage incidence of each of the four types, in 240 consecutive dissections, is indicated.

Figure 1a, nerve between heads of muscle; 1b, nerve under humeral head, ulnar head missing; 1c, nerve under ulnar head; 1d, nerve through humeral head.

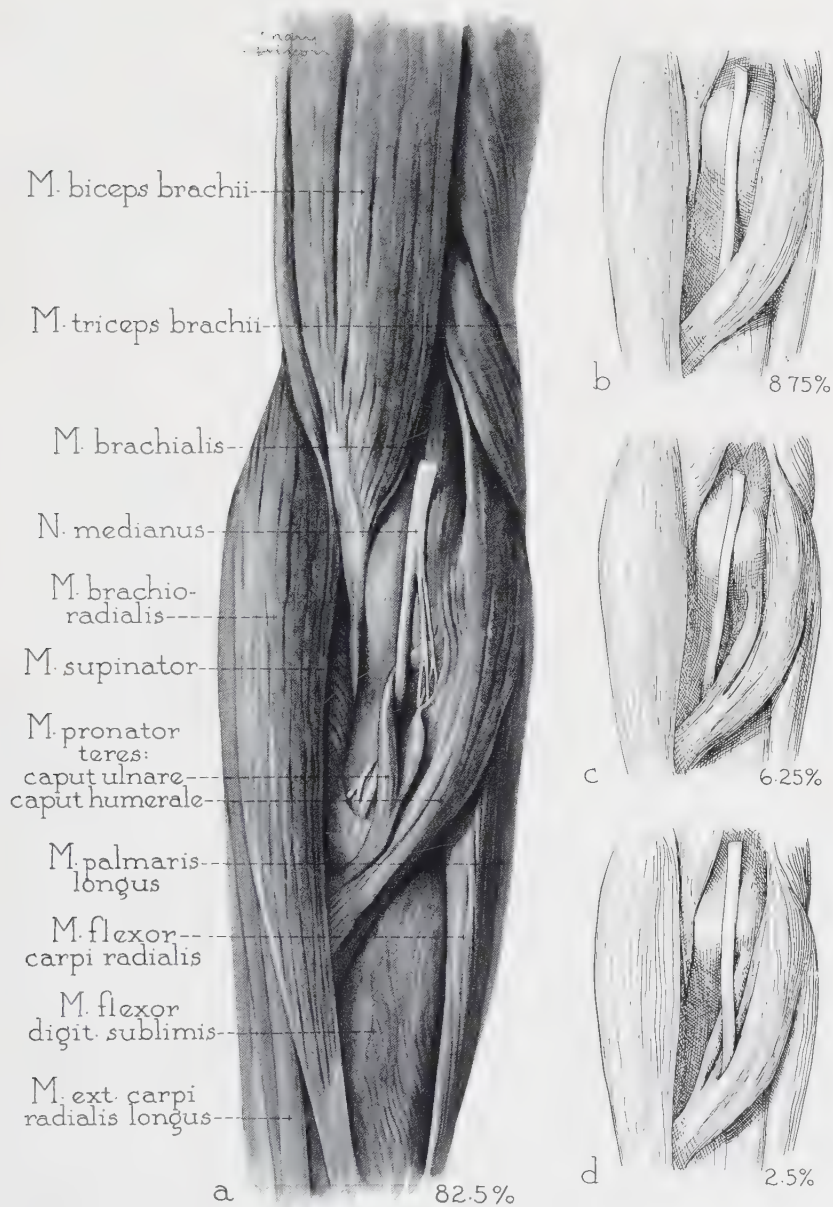


Figure 1

and Barrett ('36). Grosjurin also described an arm in which the nerve passed between such a third head and the humeral head. Ferner ('37) reported upon a specimen in which the median nerve was superficial to the pronator teres muscle; similar anomalies were described earlier by Walsh and by Gruber (Schafer and Symington, '09). Salmon and Granjon ('33) described a case in which the nerve bore no relation to the muscle pronator teres, but entered the forearm by passing beneath a portion of the flexor digitorum sublimis muscle.

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NOTE ON THE THYROID AND PARATHYROID GLANDS OF THE VIRGINIA DEER

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THREE FIGURES

The histological structure of the thyroid and parathyroid glands of the Virginia deer has been investigated, with particular reference to autofluorescence and fat. Search of the literature reveals a complete absence of histological data upon the glands in this species. Furthermore, our knowledge of autofluorescence of the mammalian thyroid and parathyroid is meager, being confined to the observations of Hamperl ('34) upon human material.

From a freshly-killed male specimen (taken in southern Massachusetts in November; 'spikehorn'; estimated age 1½ years) of northern Virginia deer (*Odocoileus virginianus borealis*), the thyroid and parathyroid glands were removed and fixed in 10% neutral formalin. Fairly complete autopsy showed no gross pathology.

For studies of autofluorescence frozen sections, cut at 10 μ , were mounted unstained in C.P. glycerine (which is non-fluorescent). The microscope employed for these observations was the Leitz Ultropak, using the monocular attachment, 12X ocular, and 11X dry and 23X water immersion (without water cap) objectives. The light from an intense electric arc was passed through a polished Corning filter, Corex G986A, and concentrated by the bull's-eye furnished by the manufacturer. Formalin has proved to be the fixative of choice for studies of autofluorescence; and the observations were made within a week after the death of the animal, since even with formalin considerable alterations in fluorescence may result from long immersion (Haitinger, '34; Hamperl, '34).

For the demonstration of fat, frozen sections ($10\ \mu$) were stained for 5 minutes in Sudan III (saturated solution in 70% alcohol with 1% KOH), and mounted in glycerine. For routine histological study the tissues were embedded in paraffin (parathyroid) or celloidin (thyroid). The thyroid was so hard after paraffin embedding that sectioning was impossible. Celloidin sections were cut at $10\ \mu$, paraffin sections at $5\ \mu$. The sections were for the most part stained with hematoxylin and eosin; others were stained with hematoxylin alone or were mounted unstained.

OBSERVATIONS

Thyroid gland

Autofluorescence. With 11X objective, 12X ocular: The follicular structure of the gland is beautifully delineated in virtue of a fairly intense autofluorescence of the follicular epithelium. This fluorescence is of an ill-defined greenish-yellowish-white color. The delicate interfollicular tissue is frequently delineated as a discontinuous, thin, dark line; the dark areas frequently have a suggestive flat brown color, presumably to be ascribed to the presence of erythrocytes in the perifollicular capillaries. Without exception the follicular epithelium is sharply demarcated from the follicular contents; the apical surface of the epithelium is a sharp, even line with no perceptible irregularities. No details of cell structure can be made out, and, in terms of fluorescence (both intensity and quality), one would be forced to conclude that the follicular epithelium is made up of only one type of cell. In the majority of the follicles the intrafollicular space is essentially devoid of fluorescence, appearing dark, and uniformly so, in contrast to the well-defined epithelium. In such follicles one sees at best the faintest suggestion of a greenish-white fluorescence, presumably attributable to the colloid. There is no suggestion of 'scalloping' at the periphery. There are all gradations from this almost undetectable fluorescence of the colloid to one of fair intensity, though still much less intense than that of the follicular epithelium. In contrast to the

epithelium the colloid fluorescence has a distinctly bluish cast. Here again, almost without exception, the colloid is homogeneous and structureless in appearance. No 'scallop-ing' is detectable, but frequently a thin dark line separates the epithelium and the colloid, suggesting a shrinkage space. The follicles showing the more intense colloid fluorescence are distributed apparently at random through the gland, and seem to include follicles of all sizes, although the most striking ones are relatively large. In rather many follicles one observes a number of small, round granules of a fairly intense bluish-white or violet-white color, and standing out distinctly against the darker background. Many follicles contain only a few of these granules, some only one or two, and some a rather large number of them. Although not all of the peripheral follicles show them, it seems clear that all of the follicles containing a large number of the granules are either immediately peripheral or just subperipheral in location. The most striking follicle in this regard is immediately peripheral, and shows a patchy distribution of the granules, of varying size but never large. At a rough estimate the total area of the granules would be about one-sixth of the total area of the colloid.

With 23X objective, 12X ocular: Use of this lens serves only to confirm the above observations. No further details of the follicular epithelial cells can be made out. Follicles from which the colloid has fallen out appear entirely black compared to the distinct fluorescence where the colloid is still present. With this lens the fluorescence of the colloid in the palest follicles is much more appreciable than with the preceding lens. One obtains the impression that the intensity of the fluorescence of the colloid is probably roughly proportional to its density.

Fat. Fat droplets, well stained with Sudan III, are regularly present in the follicular epithelium (fig. 1). These droplets are quite variable in size: the smallest ones are very minute; the largest ones are of fairly good size, but

may still be designated as small. Some cells are entirely devoid of recognizable fat, but these are relatively rare. Other cells show only one or two fat droplets; however, the presence of at least several droplets is the rule. In many cells the droplets are fairly numerous, but no cells heavily

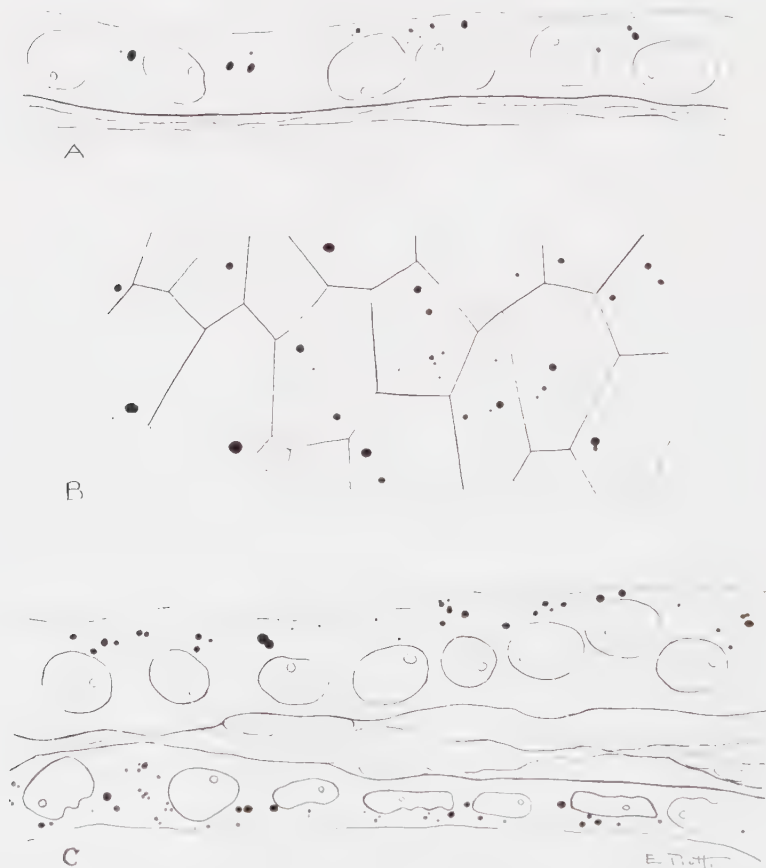


Fig.1 Fat droplets (black) in follicular epithelium of deer thyroid. From frozen sections, stained with Sudan III. Camera lucida drawings at $\times 1600$; as reproduced, $\times 1280$. A, fairly typical picture of the follicular epithelium in vertical section (drawn at one focus). B, small region of epithelium in surface view (fat droplets at all focusses included). C, epithelium of two adjacent follicles; here the fat droplets are more numerous than usual, and extend into the basal region of the cells in the lower left corner of the figure.

laden with fat are encountered. The fat droplets are usually located in the supranuclear and (to a lesser extent) paranuclear regions, and only occasionally do they extend into the basal half of the cell. From the standpoint of fat content and distribution, one would be unable to say that there was more than one type of follicular cell throughout the gland. No fat-containing cells are observed in the interfollicular tissue. The colloid is not itself sudanophilic, and no sudanophile inclusions are found in it.

General histology. The gland is remarkably compact, as can be appreciated from figure 2A, which is a cross section of one entire lateral lobe. In the absence of any large connective tissue septa, the gland is devoid of any apparent 'lobular' structure such as characterizes, for example, the human thyroid. Only small amounts of connective tissue accompany the larger vessels, and the vascular interfollicular connective tissue is quite delicate. The follicles vary widely in size; very large follicles are few in number, and there is an abundance of small follicles, as can be seen in the figure.

The follicular epithelium is for the most part low cuboidal in character (fig. 2B). The nuclei are typically oval (more or less elongated) in shape, but may be round. The cytoplasm is usually clear or faintly basophilic, sometimes neutral, and rarely eosinophilic in staining reaction. It is non-granular, but is frequently vacuolated in varying degree. Rather large, swollen, markedly vacuolated cells are sometimes found in the follicular epithelium. They occur for the most part singly, rarely in groups of two or more. Their nuclei show no degenerative change. The cuboidal epithelium varies somewhat in height, but columnar epithelium is nowhere observed. Sometimes, on the other hand, the follicular epithelium is quite flattened, with moderately to markedly elongated nuclei. Many nuclei are rather irregular in shape, and may be, in addition, somewhat more deeply staining than usual. However, despite intensive search for them, no obvious Langendorff cells could be identified anywhere in the gland. There is likewise a complete absence of any

recognizable 'interfollicular' cells suggestive of the 'para-follicular' cells of Nonidez ('33). Careful study of stained and unstained sections reveals a complete absence of pigmented material in the epithelium, stroma and colloid.

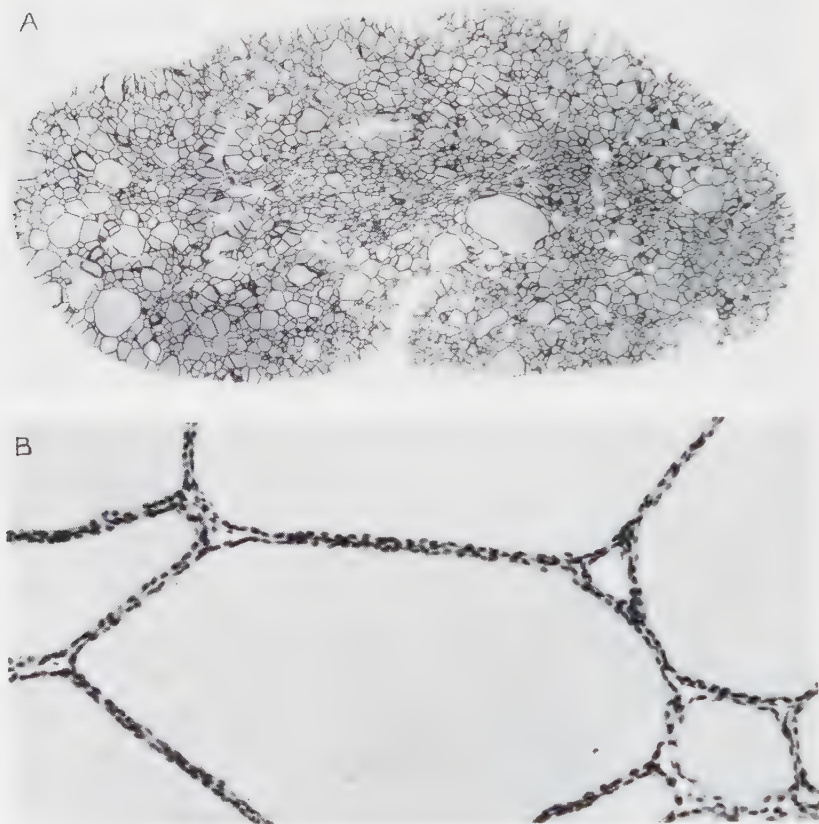


Fig. 2 Deer thyroid. A, low-power photograph of transverse section through one entire lateral lobe of the gland, from which the capsule has been stripped; H. and E., $\times 10$; see text. B, higher-power photograph of a small portion of the above section; $\times 200$; see text.

The density of the colloid varies somewhat, but the picture is surprisingly uniform throughout the gland. There is no peripheral 'scalloping' of the colloid, and isolated clear vacuoles, such as are seen in figure 2B, are present in only

relatively small numbers. The colloid is typically eosinophilic, sometimes neutral, and never frankly basophilic in staining reaction (see below). No intrafollicular accumulations of erythrocytes are observed. The colloid is usually entirely free of cells of any type, but may contain one or two desquamated, degenerating cells. In a single instance, in a rather large follicle, there are rather numerous cells scattered indiscriminately throughout the colloid. The well-preserved cells are predominantly large mononuclear elements, with markedly vacuolated cytoplasm. These cells are so similar in character to the large, vacuolated cells described above in the follicular epithelium that it seems wholly justifiable to interpret them as desquamated epithelial cells. In addition, there are a few intact polymorphonuclear neutrophils. Finally, a number of naked nuclei are present, and these show varying degrees of degenerative change. The colloid in this follicle shows no other abnormality, and takes the eosin as usual. No erythrocytes could be found. The follicular epithelium is apparently normal, although in one small region it is quite flattened, with markedly elongated nuclei.

As described above, under the fluorescence microscope one observes, in rather many follicles a number of small, round granules of a fairly intense bluish-white or violet-white color. It is naturally of interest to determine whether there is any morphological equivalent of these granules in ordinary histological preparations. As shown in figure 2B, the colloid consistently exhibits a delicate, irregular 'pattern' or lack of homogeneity, which obviously cannot be responsible for the fluorescent granules seen in only a minority of the follicles. Free cells are virtually absent from the colloid, and can likewise be ruled out as a basis for the fluorescent pictures obtained. Careful scrutiny of the sections reveals, in some of the follicles, a varying number of small masses which are distinctly (but never markedly) basophilic in staining reaction. The follicles containing these masses are predominantly peripheral, but at times subperipheral, in location. The density of the colloid is usually lighter than average, and may be

markedly so. The masses themselves are variable in size, but never large. They are typically round or oval in shape, but may be rather elongated. In a given follicle one may find anywhere from one to ten or more such masses; the presence of several is the rule; no follicle heavily laden with them was observed. The masses are usually scattered more or less at random in the colloid, but in one striking instance they are all located peripherally, adjacent to the follicular epithelial cells. Some of the larger masses are not homogeneous, having a vacuolated appearance. In the absence of any other recognizable structure in the colloid, it is concluded that these basophilic masses are equivalent to the 'granules' observed in fluorescence studies.

Parathyroid gland

Autofluorescence. The fluorescence of the parenchymal cells is very pale indeed; no structural details can be made out, and, on the basis of fluorescence, there is no evidence of more than one type of cell. In contrast to the parenchyma, the larger connective tissue septa stand out quite well with a fairly intense fluorescence of a bluish-white quality. At high power the more delicate septa can be visualized as distinctly more fluorescent than the parenchyma.

Fat. The parenchymal cells, with fair uniformity, contain a number of fine fat droplets scattered in their cytoplasm. These droplets vary somewhat in size, but are predominantly quite small. Some cells are entirely devoid of recognizable fat, but these are relatively rare. Cells heavily laden with fat are nowhere encountered. The distribution of the fat droplets is so variable that it is impossible to arrive at any conception of a cellular polarity. On the basis of fat, there is no evidence of more than one cell type in the gland.

General histology. In architecture the gland is intermediate between the 'compact' and 'lobulated' types as usually designated. A number of connective tissue septa of moderate thickness penetrate irregularly into the gland, but complete encirclement of portions of the parenchyma is rarely observed.

The connective tissue within the gland is entirely devoid of fat cells. A fairly typical area of the compact parenchyma is illustrated in figure 3. The vascular bed of the gland is very extensive indeed; the cell cords are seldom more than two cells thick, and there is every reason to believe that each cell abuts upon a capillary wall. As is usual in histological preparations, many of the capillaries are partially or entirely collapsed. Where they are naturally injected with

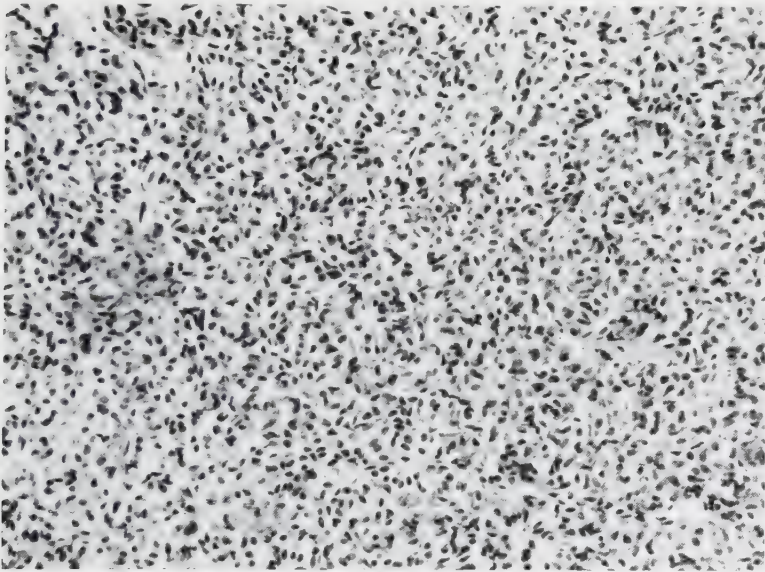


Fig. 3 Deer parathyroid; H. and E.; $\times 200$; see text.

blood, it is obvious that their caliber is unusually large (two or more erythrocyte diameters in width). In the American literature it is common to designate such capillaries 'sinusoids' (Minot, '00), although this term has been rejected for the parathyroid by Bargmann ('39).

The gland is apparently made up of a single cell type, which is homologous with the 'principal' cell of the human parathyroid. The nuclei are typically oval, with two or more sharply-defined nucleoli. The basic cytoplasm takes no

stain and is remarkably characterless. A common finding is the presence of numerous small basophilic granules in the cytoplasm. In view of the possibility that they may represent a secretion antecedent in the cell, these granules will be studied further when more adequate material can be obtained. A few cells exhibiting degenerative change are encountered. Furthermore, one occasionally observes a cell with a mildly eosinophilic and somewhat vacuolated cytoplasm. However, these cells are for the present interpreted as simple variants of the basic cell type. No true 'oxyphile' cells, comparable with those in man, can be identified anywhere in the gland. A follicular arrangement of the cells is sometimes observed, but the central cavity is uniformly devoid of colloid. There is no pigment in either the epithelium or stroma of the gland.

DISCUSSION

Of particular interest are the present observations upon autofluorescence, in comparison with those of Hamperl ('34) upon the human thyroid and parathyroid. In the deer the fluorescence of the parathyroid cells is very pale indeed, and no structural details can be made out. In man Hamperl finds the cells devoid of fluorescence except for the presence (in the parathyroids of older individuals) of fat-containing 'wear-and-tear' pigment (Abnutzungspigment), which exhibits a marked golden-yellow fluorescence.

In the thyroid of the deer the follicular epithelium is characterized by a fairly intense autofluorescence of an ill-defined greenish-yellowish-white color. According to Hamperl the follicles of juvenile human thyroids show no autofluorescence, and only once did he observe the apical portion of the cells to fluoresce somewhat more brightly than the rest of the protoplasm. The appearance of the epithelium is modified, in older individuals, by the presence of granular lipoids and Abnutzungspigment, which have a yellow-brown fluorescence. In the deer the colloid is usually almost devoid of fluorescence (a faint greenish-white in color), but all gradations are en-

countered up to a fluorescence of fair intensity, when the colloid has a bluish cast. In young people Hamperl finds the colloid essentially non-fluorescent, but in older individuals the picture is quite variable. Many follicles are completely filled with bright blue fluorescent colloid, which from control staining he identifies as the so-called basophilic colloid. In addition, the colloid contains numerous small granules (possessing a yellow color of their own, sudanophile, and not wholly dissolved in paraffin sections), which show a very marked fluorescence, in tones between brilliant yellow and blue-white. He considers these granules to consist, in part at least, of fat-containing pigment. Finally, he finds in the colloid rather large round masses, of a blue-white fluorescence, which he interprets as desquamated and decomposing epithelial cells. As described above, the colloid in some follicles in the deer contains a number of small, round granules which exhibit a fairly intense bluish-white or violet-white fluorescence. Study of ordinary histological preparations establishes the identity of these fluorescent granules with small, moderately basophilic masses in the colloid. From their character these masses are presumably colloid itself, in a particular state of aggregation. Thus, the present findings are in agreement with Hamperl's observation that basophilic colloid in man regularly shows a bright blue fluorescence.

The presence of sudanophile fat in the cells of the thyroid and parathyroid is in keeping with the available data upon man and other mammals (Bargmann, '39). It tends in general to increase in amount with increasing age. In the absence of oxyphile cells in the parathyroid, the deer is similar to other mammals below the primates, although Levine ('28) has established their presence in cattle.

SUMMARY

The histological structure of the thyroid and parathyroid glands of the northern Virginia deer (*Odocoileus virginianus borealis*) has been investigated, with particular reference to autofluorescence and fat. The present observations upon

autofluorescence are compared with those previously reported for man by Hamperl. Bright-blue fluorescent granules in some of the thyroid follicles are identified with small, moderately basophilic masses of colloid. The parathyroid is composed of a single cell type, homologous with the 'principal' cells in man. No oxyphile cells are present. Fat is typically present in the parenchymal cells of both glands, but is never large in amount.

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THE MICROSCOPIC APPEARANCE OF CONTRACTIONS OF STRIATED MUSCLE

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TWO PLATES (EIGHT FIGURES)

INTRODUCTION

Exner (1887) described what he called 'Schrumpfkontraktionen.' These were regions of muscle fibers which appeared to be in a contracted state in that the cross striations were more closely approximated than elsewhere (fig. 1). Dahlgren ('33) described nodes of contraction occurring in the tail of the 'torpedo' (*Tetronarce occidentalis*) in striated muscle (fig. 2). These are seen to be nodes marked by densely staining myofibrils and which are not limited by the usual architecture of striated muscle. He remarked on the resemblance of these nodes to those occurring in the contractions of smooth muscle (as illustrated by McGill, '07 and '10 a and b).

It was found possible to produce this effect in the striated muscle of the stomach of the common spiny dogfish by faradic stimulation during fixation of the tissue with Bouin's fluid (fig. 4). These bands of contraction were noted to consist of dark staining areas on adjacent myofibrils, which could readily be seen under high power to be increased in diameter. The manner in which nuclei (central in this primitive striated muscle) caught in these nodes are compressed serves to prove that this increase in diameter is real. The behavior of nuclei between nodes shows that shortening has taken place.

On the basis of the similarity of these nodes to those appearing in the normal contraction of smooth muscle (fig. 3), and the usual form of contraction observed in smooth muscle,

the author felt that these nodes represented the anatomical basis for the phenomenon of contracture (Meneely, '33).

Striated muscle is capable of a type of response which differs from the propagated wave of contraction which obeys the all or none law, is relatively quick and may summate in a prolonged response with tetanic stimulation. This second method of contraction (called contracture) is slower, non-conducted (Biederman, 1895), similar in its physiology and chemistry to the usual response of striated muscle (Gasser, '30; Bethe, Northrup and Huf, '31); Hartree and Hill ('24 and '28; Furusawa and Hartree, '28; and Meyerhof and Lohman, '25). These contractures tend to appear low rather than high in the phylogenetic scale (Wachholder and Ledebur, '30; Wachholder, '30; and Freund and Ruchert, '30).

Gasser ('30) reviewed the literature of contracture and clarified the definition of the word: first, some part of the normal contractile mechanism must be involved; second, the changes must be reversible; third, tetanic contractions are excluded. Many contractures are characterized by a reversible and an irreversible phase.

Preliminary experiments. Hurthle ('31) described an apparatus whereby he fixed muscles while attached to a kymograph. The apparatus used by the present author is essentially the same as that used by Hurthle, consisting of an arrangement whereby an excised muscle may be supported and stimulated in such a manner as to allow the substitution of various fluids bathing it. There is one important difference. Hurthle used isometric recording, while in these experiments isotonic recordings were made.

It was found that ordinary fixatives were not useful in the quantitative study of striated muscle contractions but were satisfactory for the purpose of these experiments. Upon immersing the muscle in fixing fluid, while tetanizing it, the muscle relaxed considerably before the fixing fluid could act.

These early experiments were done on either the gastrocnemius, the semi-membranosus, or the semi-tendinosus of *Rana pipiens*. The muscle was fixed with Bouin's solution

while being stimulated with a faradic current. The appearance of the heavy, wavy bands traversing the fibers was characteristic. The myofibrils in the region of these bands were swollen with no regard to the cross striations of the fiber (fig. 5). The swelling extended through several sarcomeric units and took the stain more deeply. This phenomenon never occurred in muscles which were not stimulated in a manner to evoke contracture. This method was, however, not satisfactory. The fixed and stained material, while showing fine detail and permitting prolonged study of the nodes, was not adequate for study of the nature of the process of formation and relaxation. The preparations also might contain artifacts.

For these and other reasons the phenomenon was studied *in vivo*. This is possible by the following method:

A box is prepared as follows: The inverted cover of a box of $3\frac{1}{4}$ inches $\times$ $4\frac{1}{4}$ inches photographic plates serves very well. An aperture a few centimeters square is made about two-thirds of the length of the box in the middle with respect to the breadth. A 3 inches $\times$ 2 inches histological slide is glued over the opening, and a cube of glass, 1 cm. on edge is cemented with Canada balsam to the slide, inside the box. Wax is then poured into the box to within 2 to 3 mm. of the top of the glass cube.

The dissection is most conveniently made in a small tray with a half centimeter of wax in the bottom. The head of the frog is seized with a clamp in such a manner that one of the jaws of the clamp is between the jaws of the frog as far posteriorly as they will admit, and the other above the head. The clamp is firmly closed so as to crush the tissues between the jaws and destroy the brain at a point somewhere in the medulla. Leaving the clamp in place to prevent bleeding, the head is cut off anteriorly to the clamp. The lower jaw thus remains undisturbed. The tongue is now seized at the tip with delicate hemostats and the frog is placed on its back in the dissecting tray with a gentle stretch exerted on the tongue to keep it everted. The thick pad on the surface of the tongue is now presented and the tray may be filled with

Ringer's solution. With a pair of fine forceps, a bit of the epithelium in the midline is elevated and a median incision in the pad is made by means of a pair of iridectomy scissors. The flaps are pinned back and the tissue underlying gently divided down to the retrolingual lymph sac. When the sac is opened, the preparation is complete. Care must be taken not to interfere with the arteries, which lie laterally in the tongue, for bleeding is ruinous to a good preparation. The anastomosis between these two lateral arteries must be dealt with by seizing it with two fine forceps and tearing it between them, holding the ends until they will not bleed. The preparation is rinsed with a gentle jet from a wash bottle of Ringer's solution and the pins removed. The frog is then put belly down in the box, described above, and the block of glass projecting above the wax is made to come up through the above described incision. The tongue is pinned out so that the membrane lies smoothly in direct contact with the glass surface. Two pins driven through the lower jaw of the frog immobilize the preparation. The hemostat used to evert the tongue is now removed. The box should fit the mechanical stage of the microscope. The membrane is lighted from below as with a mounted section. Observation is best made with a $\times 40$ water immersion lens. The box is kept well filled with Ringer's solution. A micromanipulator is used to apply microelectrodes to the fiber, or to approximate a micropipette charged with a drug (fig. 8).

OBSERVATIONS

The micro-pipette was brought near a selected fiber so that a few cubic millimeters of 1:100 caffeine in Ringer's solution might flow over it. After a short latent period there was evidence of activity. The fiber moved gently on its long axis, the nice arrangement of the cross-striation being disturbed as the fiber tugged back and forth. Soon it was apparent that a node was forming in which the striations were pressed to-

gether so closely that it was difficult to make them out. At this juncture the fluid about the fiber containing the caffeine was sucked back into the pipette and there was drawn over the affected region a flow of fresh Ringer's solution to dilute and wash away the drug. Reversal of the process of contraction immediately set in and very soon the node vanished, and the various constituents of the muscle resumed their normal arrangement. The same result followed the application of a constant current. The effect was completely reversible and repeated as often as desired (fig. 6).

During the time when the node of contracture was at a maximum it was possible to study its structure. The sarcous membrane was wrinkled over the node. The adjacent regions on the fiber were put on the stretch and in these regions the distance from one 'Z' line to the next was increased. In the node itself, the cross-marking was still apparent, but crowded to a remarkable degree. The strain on the fiber was so great that it sometimes ruptured. The myofibrils were apparent in all parts of the fiber, right through the node, in which they were of larger diameter than elsewhere. The nodes remained local and were not propagated.

It must be emphasized that this is not the usual response of striated muscles. During these experiments, ample opportunity was afforded to make this certain. For, in manipulating the preparation, fibers were frequently seen to twitch. In this case, the rate of reaction, and the propagation of the contraction, as well as its appearance, are quite different from those of the contracture.

If one persists in the stimulation of a fiber, or stimulates more vigorously, a new response becomes apparent. This is a response of injury. The cross-markings within the node grow fainter. The constituents of the node flow together and in an instant the node becomes homogeneous and refractive. The process is now no longer reversible. Such fibers give the appearance of Zenker's hyaline degeneration of muscle (fig. 7).

CONCLUSION

Fixed and stained material is presented to illustrate the presence in striated muscle of a state resembling in appearance the usual 'nodes' of contraction in smooth muscle.

A modification of the retrolingual membrane preparation is described. With this it is possible to produce in a living striated muscle fiber a type of response which differs from the 'twitch' response. It is a contraction, and is associated with myofibrillar activity and probably due to this. It is slow compared to the twitch response and is not propagated. The characteristic myofibrillar changes occur without limitation by the subdivisions of the sarcomere. It is reversible up to a point, after which an irreversible change takes place which may be recognized as Zenker's hyaline degeneration. It has, in the reversible phase, a striking similarity to the usual contraction of smooth muscle. It is an adequate anatomical basis for the type of response of striated muscle known as 'contracture.'

The author wishes to express his gratitude to those who have helped him with this study: Dr. Ulric Dahlgren of the Department of Biology of Princeton University in whose laboratory the work was begun, Dr. Herbert Gasser who gave the author the opportunity to continue in the laboratories of the Department of Physiology of Cornell University Medical College, Dr. Harold Grundfest who demonstrated the dissection of the retrolingual membrane, and Dr. Harold Wolff and Dr. George Corner who helped in the preparation of the manuscript.

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PLATE 1

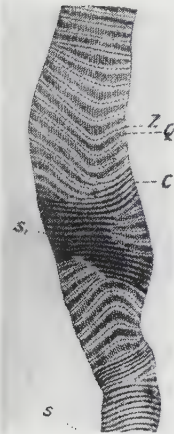
EXPLANATION OF FIGURES

1 Striated muscle fiber from the orbicularis palpebrum muscle killed in Muller's fluid, showing the 'Schrumpfkontraktionen' Exner (1887).

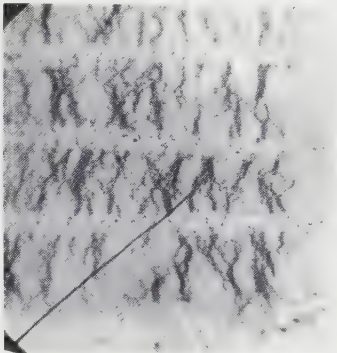
2 Striated muscle from the tail of the Torpedo Tetronarce occidentalis, showing 'x' contractions. Doctor Dahlgren's preparation (Dahlgren, '30) iron haematoxylin, $\times 300$ and $\times 1000$.

3 Smooth muscle from the gut of the cat, showing the characteristic appearance of the usual node of contraction of smooth muscle. (Doctor Dahlgren's preparation) iron hematoxylin, $\times 500$.

4 Striated muscle from the cardiac end of the stomach of the common spiny dogfish, *Squalus acanthias*, showing bands of contraction. The nuclei are central and the striations nearly invisible in this primitive muscle. (The striations may be clearly seen in glycerine mounts.) Compression of the nuclei and 'coiling' are readily seen. Haematoxylin and eosin, $\times 500$.



1



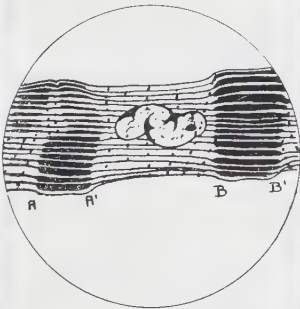
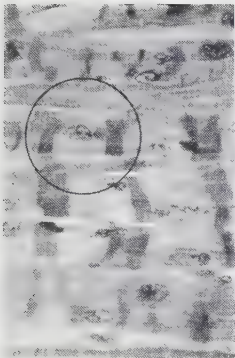
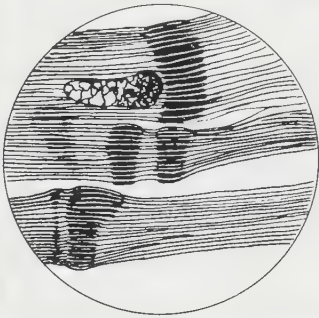
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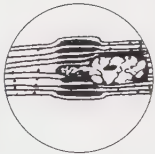
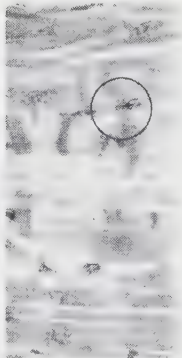


PLATE 2

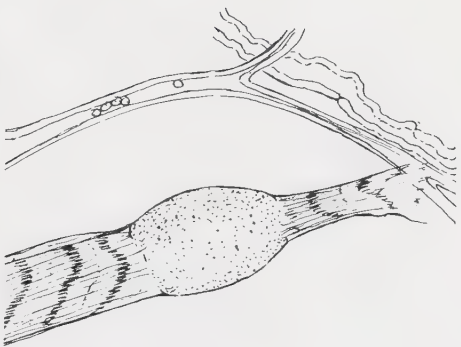
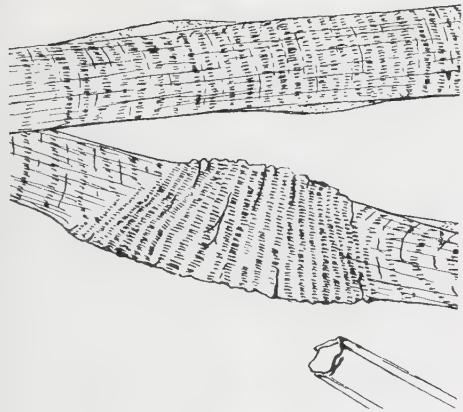
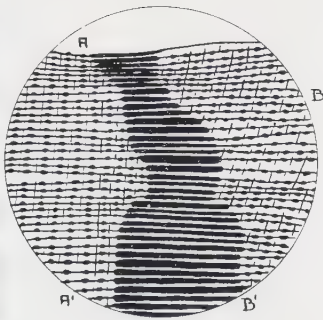
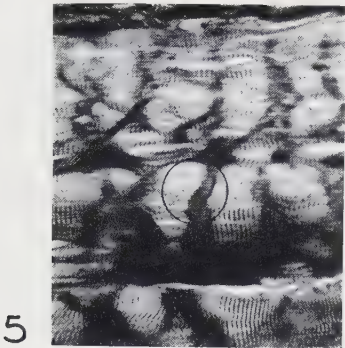
EXPLANATION OF FIGURES

5 Striated muscle from the gastrocnemius of the frog, *Rana pipiens*, fixed with cold Bouin's fluid while tetanizing. Haematoxylin and eosin. $\times 600$. In the circled node of contracture, the myofibrilli are thicker and take the stain more deeply. Shortening has taken place, for A-A' and B-B' were parallel sarcomeres.

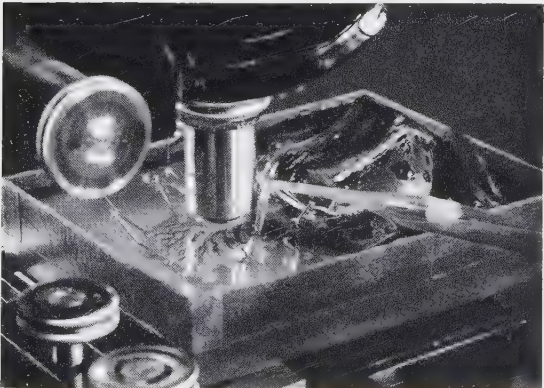
6 A drawing of a node produced by spraying the fiber with caffeine solution (1%) by means of the micropipette. This is a fully reversible reaction.

7 A node showing the irreversible phase, and which resembles Zenker's 'hyaline degeneration' of muscle. The fiber shows the effect of strong tension and has broken.

8 Retrolingual membrane preparation as used in this study.



6



7

8



STUDIES ON THE REPRODUCTIVE SYSTEM OF THE ALLIGATOR

V. THE EFFECTS OF INJECTIONS OF TESTOSTERONE PROPIONATE IN IMMATURE ALLIGATORS

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ONE FIGURE

INTRODUCTION

In the author's previous study of the effect of injections of testosterone in recently hatched female alligators (Forbes, '38b), it was reported that the experimental treatment resulted in development and hypertrophy of the oviducts of the females and in the growth of the penis of a single experimental male. The present study was undertaken in the effort to confirm the latter result and to observe the effects, if any, of an injected androgenic substance on the gonads of immature male and female alligators. Somewhat older animals were used in this experiment, and testosterone propionate was substituted for testosterone since numerous investigators have shown that the esterified compound has, in general, a greater stimulating effect than does testosterone alone.

MATERIAL AND METHODS

The thirty-four alligators which were used were maintained in a single cage, the control group (seventeen animals) being securely isolated from the experimental group (seventeen animals) by an intervening partition. The animals were fed plentifully with raw beef liver and muscle.

Testosterone propionate in a weakly alcoholic aqueous solution was injected intraperitoneally, 6 days a week. The injections, begun when the animals were aged approximately 17 months, were continued over a period of 11 weeks. Each in-

jected animal surviving until the end of the experiment had received a total of 10 mg. of the hormone. The daily amount administered was gradually increased so that the daily dose during the last weeks of the experiment was more than three times that given during the first weeks. One control and four injected animals died during the course of the experiment.

Two days after the last injection all surviving animals were killed, and the reproductive organs were fixed in Bouin's fluid. Serial sections were cut at 10 μ and stained with Mayer's acid hemalum and eosin.

The testosterone propionate used in this study was generously supplied by Dr. Erwin Schwenk of the Schering Corporation.

RESULTS

Only observations based on animals surviving until the end of the experiment will be reported.

A. Macroscopic observations

Autopsy of the sixteen controls killed at the end of the experiment showed that nine were males and seven were females. Of the thirteen injected animals sacrificed at this time, seven were males and six were females. The oviducts of the experimental females showed a gross increase in length and diameter and an irregular conformation similar to that described and illustrated in the earlier report (Forbes, '38 b). The penes of the experimental males showed uniformly a striking hypertrophy as compared to those of the control males. In addition, all of the clitorides of the experimental females were definitely larger than those of the control females (see figure). Hypertrophy of the clitorides was an interesting result not obtained in the previous experiment. As indicated in the figure, a typical control clitoris was smaller than a control penis, while an experimental clitoris was larger than the latter, and the experimental penis was largest of all. The growth of the phallus in both male and female experimental animals was so great that the tip of the organ pro-

truded from the vent since the cloaca, not visibly stimulated by the hormone, had failed to keep pace in its growth with the hypertrophy of the phallus..

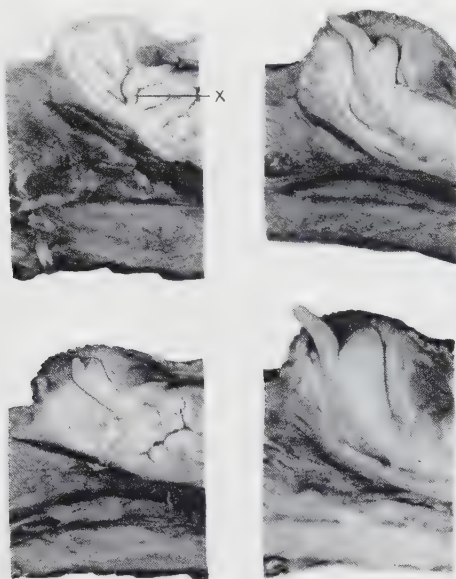


Fig. 1 This figure shows typical median sagittal sections through the cloacal regions of a control female (upper left), an experimental female (upper right), a control male (lower left), and an experimental male (lower right). The cloacal opening is uppermost in each case. Note relative sizes of penes and clitorides. $\times 1\frac{1}{2}$.

B. Histological observations

1. *The gonads.* The right and left gonads of six control males, six control females, six experimental males, and six experimental females were removed for complete transverse serial sectioning.

Careful study of the sections revealed no significant qualitative differences between control and experimental gonads.

The possibility of quantitative changes in the ovaries and testes was investigated by cutting out and weighing camera lucida outline tracings on paper of regularly spaced sections

from each of the gonads studied. This method indicated quite accurately the relative volumes of various gonad components. Because of a wide variation in individual values for each type of component within both control and experimental groups, such quantitative changes as seemed to have been induced did not support definite conclusions and could only be viewed as suggestive. Of the various components, testicular germinal epithelium, ovarian cortex, and typical ovarian medulla seemed not to be materially affected. The ratio of average testicular medullary volume (i.e., the volume of the testis exclusive of the germinal epithelium) of the control males to that of the experimental males was as 1:1.38. The ratio of average volume of medullary rest (an area of solid medullary tissue which lies at the posterior end of the ovary and lacks a cortex) of the control females to that of the experimental females was as 1:0.79. (See Forbes, '39, for a fuller description of the components of the gonads of immature alligators.)

These data would seem to suggest that under the conditions of the experiment the testosterone propionate exerted a differential effect on the various parts of the ovaries and testes of the injected animals. Evidence sufficient to support definite conclusions, however, is not yet available.

2. *Observations on the adrenals, mesonephroi, and gonoducts.* Histological comparison of the adrenals, mesonephroi, and mesonephric ducts of control and experimental animals revealed no significant differences.

Grossly, as already indicated, and microscopically, the changes in the oviducts of the injected female animals were quite similar to those observed in the younger series of alligators injected with testosterone (Forbes, '38 b). The oviducts of the experimental females of the present series showed evidence of considerable hypertrophy anteriorly in that the more cephalic levels were thrown into irregular folds while the lumina were increased in diameter and the mucosa, normally a simple columnar epithelium, was increased in height and was so hyperplastic that it appeared rugose and

pseudostratified. More posteriorly the duct lumen and mucosa were more nearly normal, but a diffuse stratum of smooth muscle, not seen in the controls, had made its appearance in the connective tissue wall of the oviduct. The smooth muscle layer was best developed in the region of the mesosalpingeal attachment.

A search was made in the sections of male tissues for müllerian duct vestiges. In one control and in three experimental animals there was found a short, single or double wave-like fold of connective tissue invested with peritoneum; these folds arose from the dorsal body wall, just lateral to and at the level of the mesonephros and in a position homologous to that of the root of the mesosalpinx in the female. It is conceivable that these structures may have represented highly regressed müllerian duct vestiges although they were provided neither with epithelial cells nor lumina. In no case were the suspected areas as well developed as the most primitive müllerian duct remnants observed in the normal males of an earlier experiment (Forbes, '38 a).

DISCUSSION

For reasons already indicated, the data regarding the possible action of testosterone propionate on the gonads are scarcely adequate to support any general conclusions. Nevertheless, the apparent stimulating effect of the hormone on the testis proper is suggestive, particularly in the light of Risley's parallel observation ('37) that the injection of testosterone propionate into turtle eggs was followed by a hypertrophy of the gonadal medullary tissues of the embryos. The failure of the ovarian medulla of the alligator to respond to stimulation may well be ascribed to the highly regressed condition of the medulla in animals of the age used in this experiment.

The individual variation in the volumes of the various gonadal areas has been observed by the author in previous experiments and is believed to be quite normal. It is not unlikely that such variation is more common in biological material than is generally suspected.

Oviduct hypertrophy and a lack of response of the mesonephros and its duct following the administration of testosterone to recently hatched alligators was discussed in an earlier paper (Forbes, '38 b); the use of older animals and an esterified form of the hormone did not significantly alter the results in respect to these structures.

The hypertrophy of the clitorides as well as of the penes was an interesting result. In the earlier experiment with male hormone only the penes responded. It seems probable that the response of the female phallus was due either to a lower threshold of reactivity in the older animals, or to the greater potency of testosterone propionate as compared to unesterified testosterone, or to both these factors. The injection site (intraperitoneal) and the total amount of hormone administered to each individual (10 mg.) were the same in the two experiments, and the duration of the injection periods (13 weeks, 11 weeks) was nearly so. Hypertrophy of the clitoris of the alligator as a result of treatment with androgenic material is in agreement with the results obtained by administration of androgens to immature female ducks (Bulliard and Ravina, '38) and to various prepubertal mammals.

SUMMARY

Each of seven male and six female alligators received a total of 10 mg. of testosterone propionate by intraperitoneal injection over a period of 11 weeks. The experimental animals and the nine male and seven female control animals were approximately 17 months of age when injections began. The oviducts of the injected females showed a uniform and marked development and growth, and the penes and clitorides of injected animals of both sexes were strikingly hypertrophied. The mesonephroi and mesonephric ducts were not affected in either sex. Histological findings suggested some influence of the hormone on the gonads, but the evidence was not conclusive.

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THE EFFECT OF PROGESTERONE ON THE MOUSE OVARY AS INFLUENCED BY GESTATION ¹

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TWO PLATES (ELEVEN FIGURES)

Beard (1897) and Prenant (1898 a, 1898 b) were the first to put forward the theory that the corpus luteum of pregnancy is in some way responsible for the fact that no follicles mature and no corpora lutea are formed as a rule during the gestation period. This conception appeared to receive support from the experiments of Hill and Parkes ('32), who found that the corpora lutea of gestation tend to involute if a second set of corpora lutea is produced in pregnant rabbits by the administration of a gonadotrophic extract prepared from the urine of pregnant women. The authors concluded that the newly formed corpora "appear to exercise an adverse influence on the existing corpora lutea."

Numerous investigators have attempted to come closer to an understanding of the action of corpora lutea on the remaining ovarian tissue by studying the ovarian changes induced by the administration of corpus luteum implants or extracts. The findings of the early investigators were inconclusive because they used crude preparations which contained little if any actual corpus luteum hormone (Pearl and Surface, '14; Herrmann and Stein, '16; Corner and Hurni, '18; Haberlandt, '22; Cocchi, '32; Gostimirovic and Krämer, '32).

Even the observations of those investigators who used partially purified active corpus luteum preparations are

¹The expenses of this investigation were defrayed in part through a grant in aid received from the Schering Corporation.

contradictory. Thus Bates et al. ('35) claimed that corpus luteum hormone exerts an adverse influence on active mature fowl ovaries. Papanicolaou ('24, '26) noted that in the ovaries of mature guinea pigs, treatment with a lipid fraction of corpora lutea causes a delay in ovulation accompanied by the disappearance of corpora lutea and marked follicular atresia. Macht et al. ('29) found that treatment with a corpus luteum extract decreased the number of mature follicles and caused follicular atresia in the guinea pig. In the mouse, Patel ('30) noted that a corpus luteum hormone-containing extract may lead to continuous anestrus without any histological evidence of an ovarian disturbance. Several authors noted that in rabbits, corpus luteum extracts inhibit ovulation and corpus luteum formation following mating (Kennedy, '25; Mahnert, '30; Fels, '34; Makepeace et al., '36, '37). Knaus ('24) claimed that if mature female rats are treated with a corpus luteum extract, they remain sterile and histological studies of their ovaries indicate that the sterility is due to the fact that although maturation of the follicles is not interfered with, ovulation is prevented by this treatment. Hisaw et al. ('28) also reported the presence of large follicles and the absence of recent corpora lutea in the ovaries of rats treated with an active corpus luteum extract. Gley ('28 a, '28 b, '28 c), on the other hand, claimed that such treatment leads to disappearance of mature follicles though corpora lutea remain plentiful, while Abe ('31, '32) stated that both corpora lutea and follicles disappear under the influence of corpus luteum hormone treatment in the rat.

The observations of recent authors who used crystalline progesterone are likewise somewhat contradictory. Selye et al. ('36) administered 4 mg. of synthetic progesterone daily to normally cyclic rats. They noted immediate cessation of the vaginal cycles and autopsy on the thirteenth day of treatment revealed atrophic ovaries. They concluded that progesterone inhibits the development of the ovaries. On the other hand, McKeown and Zuckerman ('37) claimed to have obtained exactly opposite results inasmuch as they believed they had

seen corpus luteum formation as a result of progesterone treatment, which would indicate that this hormone actually stimulates the ovary. It is noteworthy, however, that in this experimental series, only 1 mg. of progesterone was administered daily, that is to say, one-fourth of the dose used by Selye et al. ('36). Since our own unpublished data indicate that 1 mg. per day is not sufficient to inhibit ovulation completely in the adult rat, it seems possible that the recent corpora lutea observed by McKeown and Zuckerman in their progesterone treated animals were not formed as a result of the treatment but in spite of it. In fact, since these investigators used post-pubertal rats, we see no reason to assume that the recent corpora lutea observed in the ovaries at autopsy should have been produced by the progesterone treatment. McKeown and Zuckerman argued that their theory, according to which progesterone causes ovulation and corpus luteum formation, receives further support through the observations of Shapiro ('36), Shapiro and Zwarenstein ('37) and Zwarenstein ('37), who found that treatment with this hormone elicits ovulation in the toad, *Xenopus laevis*. It must be borne in mind, however, that in the case of this amphibian, not only progesterone but a great many other sterols have been shown by the above-mentioned investigators to cause extrusion of ova even from the excised ovary kept in vitro in Ringer's solution. In this case, the mechanism of ovulation is obviously different from the pituitary-controlled ovarian response in the rat. Dempsey ('37) and Dempsey et al. ('36) claimed that a single dose of 0.2 international units of progesterone delays the occurrence of the next expected ovulation in mature normally cyclic guinea pigs and that daily administration of 0.05 international units prevents ovulation during the entire duration of the treatment. The ovaries of these animals contained no corpora lutea but many atretic and some normal mature follicles. The authors concluded that progesterone prevents ovulation and corpus luteum formation without interfering with follicular growth. Experiments on the rabbit led Peraus ('36) to conclude that the life span of corpora lutea of pseudo-pregnancy

is not significantly altered by progesterone and Spanio ('38) observed no change in the ovaries of rabbits following seven daily injections of 1 mg. of this substance. On the other hand, pretreatment with progesterone inhibits ovulation and the formation of corpora lutea following subsequent mating as shown by Makepeace et al. ('36, '37) thus confirming the above mentioned findings of earlier investigators who had used corpus luteum extracts.

Since we believed that the dosage has much to do with the discrepancies in the results reported in the literature, we decided to repeat these experiments in mice, using relatively large doses of progesterone. We chose the mouse as an experimental animal because Selye et al. ('33 a, '33 b) found that unlike in most other animals, the involution of the corpora lutea is particularly rapid in hypophysectomized mice, a fact which was confirmed by Leblond and Nelson ('37 a). It seemed likely therefore, that if the involution of the corpora following progesterone treatment is due to an inhibition of pituitary function, it should be particularly obvious in this species.

Another series of experiments to be reported here deals with the action of progesterone on the ovary of the pregnant mouse. This appeared to be of interest since it was found that both in the rat (Selye et al. '33 c, Pencharz and Long, '33), and in the mouse (Selye et al. '34; Leblond and Nelson, '37 a, '37 b) hypophysectomy does not interfere with the maintenance of the corpora lutea of gestation from which it was concluded that the latter are probably maintained by a placental hormone and are consequently independent of the hypophysis. The work of Astwood and Greep ('38) gave important additional evidence in favor of this conception, since these authors showed that the corpora lutea of non-pregnant hypophysectomized rats may be maintained in a functional condition by rat placenta extracts. Astwood and Greep ('39) suggested terming the gonadotrophic hormone of the rat placenta 'murine cyonin.' In view of these findings, it appeared of interest to establish whether the corpora lutea of

pregnancy are also resistant to progesterone. It was felt that if the corpora lutea of the cycle involute while those of gestation prove resistant to progesterone, this would furnish further evidence in favor of the conception that the stimuli responsible for the maintenance of these two types of corpora lutea are not identical.

EXPERIMENTAL

Our first experiment was concerned with the action of progesterone on the ovaries of normally cyclic mice. For this and all other experiments reported in this paper, black mice of the Bar Harbor strain designated as C57 were used. In the first series, six non-pregnant adult females received 1 mg. of progesterone² dissolved in 0.2 cc. of Mazola oil daily by way of subcutaneous injections for 5 days. They were sacrificed on the sixth day. Each of these animals thus received a total of 5 mg. of progesterone. Seven normal adult non-pregnant controls were killed at the same time and the ovaries of all these animals were weighed and histologically examined. The first two columns of our table summarize the ovarian weights and it will be seen that the average ovarian weight in the normal group was 9 mg. and in the progesterone-

TABLE 1

Table showing the effect of progesterone on ovarian weight in pregnant and non-pregnant mice. The ovarian weights are expressed in milligrams

NON-PREGNANT UNTREATED	NON-PREGNANT PROGESTERONE TREATED	PREGNANT UNTREATED	PREGNANT PROGESTERONE TREATED
9	3	12	13
9	3	13	9
8	4	17	12
9	6	12	10
9	5	17	12
10	3	11	15
7		9	9
		17	
		14	
Average 9	4	14	11

² The progesterone was supplied by the Schering Corporation of Bloomfield, N. J., through the courtesy of Drs. G. Stragnell and E. Schwenk.

treated group, 4 mg. The ovaries of the treated animals were different from the normals even in their macroscopical appearance inasmuch as they contained no visible corpora lutea and their color was brownish yellow instead of the normal grayish pink. Histological examinations showed that while normal corpora lutea and follicles were present in the ovaries of untreated mice, mature follicles and recent corpora lutea were invariably absent in the progesterone-treated group. In a few cases, vestiges of involuting corpora were still detectable but in most ovaries, the involution of the corpora lutea was complete. Figure 1 shows a normal control ovary with well-developed corpora lutea at low magnification. Figure 2 shows the ovary of a progesterone-treated animal containing medium sized atretic follicles and involuting corpora lutea. The interstitial tissue of the ovary likewise undergoes marked alterations under the influence of progesterone treatment. As shown in figure 3, the interstitial cells and 'theca nests' in normal mouse ovaries consist of cells having a fair amount of cytoplasm. On the other hand, in the progesterone-treated ovary represented in figure 4, the interstitial tissue is very atrophic and as a result of marked involution of the cytoplasm, cell nuclei with dense chromatin are predominant in the entire field. When examined under very high magnification, it was noted that between the atrophic interstitial cells, large light cells appear in some regions of the ovary. These have a vacuolated cytoplasm and contain a certain amount of a yellowish brown pigment which gives a positive Prussian blue reaction. It seems probable that the appearance of the pigment-containing cells is the cause of the yellowish discoloration of these ovaries which was observed macroscopically. Figures 5 and 6 are high magnifications of corpora lutea from the ovaries of untreated and progesterone-treated animals respectively. It will be observed that the corpus luteum of the treated animal shows obvious signs of involution although this was the best preserved corpus luteum in the entire treated group.

In a second experimental series, seven pregnant mice were treated with 1 mg. of progesterone daily for 8 days and killed on the ninth. In all cases, treatment was begun during the second half of gestation and three of the animals delivered normal litters on the eighth and three others on the ninth day of the experiment. The remaining animal was still pregnant when killed but judged from the appearance of the fetuses, must have been very close to delivery. Although the actual date of conception was not known in these cases, it is evident that since pregnancy in the mouse is of about 21 to 22 days duration, the treatment was administered during the second half of the gestation period. For control purposes, six pregnant non-treated animals were killed on the day of delivery and three others on the twentieth day of pregnancy. The third and fourth columns in our table indicate that the average weight of the ovaries was 14 mg. in the untreated and 11 mg. in the treated group. This would seem to indicate that progesterone caused at least a slight degree of ovarian atrophy even in this series, but considering the great individual variations, it is doubtful whether the difference is significant enough to reach such a conclusion. The gross weight of the ovary during pregnancy depends almost entirely on the number of corpora lutea and it was noted that most of the animals in the treated group had fewer corpora and fewer fetuses than those of the control group. Since treatment was begun during the second half of gestation, the number of ovulating follicles transformed into corpora lutea could not have been altered by the treatment and as the size and the histological appearance of the individual corpora was the same in both groups, it seems most unlikely that progesterone exerted no effect on the ovary of the pregnant mice. Figures 7 and 8 show low magnification views of ovaries of untreated and progesterone-treated mice respectively. It will be observed that the general size of the corpora lutea is approximately the same in both instances. Figures 9 and 10 show high magnifications of the corpora lutea from these ovaries. The structure of the corpora lutea and the appearance of their cells

are not noticeably altered by the progesterone treatment. The interstitial cells were likewise not influenced by progesterone in this series.

Concerning the maturation of follicles, our results were less clear cut. In the non-pregnant, untreated animals, large follicles were often observed, while in the treated non-pregnant group only small or medium-sized follicles were seen and there was considerable follicular atresia. In the two pregnant groups, large follicles were never observed and the amount of follicular atresia was approximately the same in the treated and the untreated animals.

It should be emphasized particularly that the administration of these large doses of progesterone did not interfere either with delivery itself or with the onset of lactation on the day of delivery. This is all the more noteworthy since numerous investigators regarded the uterine contractions which extrude the fetuses and the onset of milk secretion at the end of gestation as due to the involution of the corpora lutea and the consequent drop in the corpus luteum hormone content of the blood. Although our experiments do not give any indication concerning the factors responsible for the birth mechanism or the initiation of milk secretion, they seem to indicate that if such large doses of progesterone as were given in the present series are unable to prevent delivery and the onset of milk secretion at this time, one can hardly regard these processes as due to a sudden discontinuation of corpus luteum hormone production unless one assumes that a corpus luteum hormone other than progesterone is involved. Ehrhardt and Hardt ('37) found that a total dose of one-tenth to one-half rabbit unit of corpus luteum hormone administered in the shape of an extract, suffices to terminate pregnancy in the mouse. They concluded that great caution is indicated if this hormone is to be given to pregnant women. Our experiments show, however, that even as high a total dose as 8 mg. of pure progesterone does not interfere with gestation in this species, so that there is no reason for fear of abortion if this compound is used.

SUMMARY AND CONCLUSIONS

Experiments on the adult mouse indicate that daily administration of 1 mg. of progesterone for 5 days suffices to cause corpus luteum involution and marked ovarian atrophy. In pregnant mice, even eight daily injections of 1 mg. of progesterone do not cause significant ovarian atrophy or any detectable change in the corpora lutea of gestation. Since hypophysectomy likewise causes rapid involution of the corpora lutea in the non-pregnant mouse but remains without detectable influence on the corpora lutea of gestation, it seems possible that the ovarian atrophy caused by progesterone is due to an inhibition of the gonadotrophic function of the hypophysis. We are also led to conclude that the gonadotrophic hormone of the placenta which maintains the corpora lutea of gestation is not inhibited by progesterone.

Delivery and the onset of lactation are not prevented by massive doses of progesterone. This finding is not in accordance with the assumption that discontinuation of corpus luteum hormone production is the cause of delivery at term and of the initiation of milk secretion.

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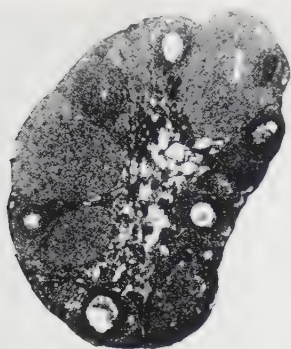
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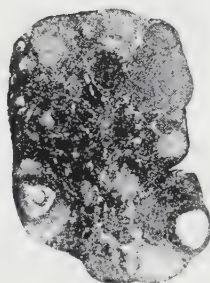
PLATE I

EXPLANATION OF FIGURES

- 1 Ovary of an untreated mouse showing normal corpora lutea of the cycle.
- 2 Ovary of progesterone treated non-pregnant mouse showing atrophic corpora lutea and atretic follicles.
- 3 Interstitial tissue in the ovary of an untreated mouse showing light cells rich in cytoplasm.
- 4 Interstitial tissue in the ovary of a progesterone treated non-pregnant mouse showing atrophy. Note the dense nuclei and the scarcity of cytoplasm.
- 5 Normal corpus luteum of an untreated non-pregnant mouse.
- 6 Involuting corpus luteum of a progesterone treated non-pregnant mouse.



1



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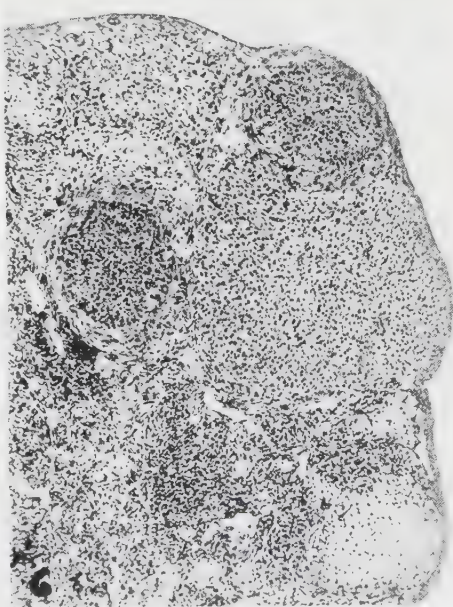
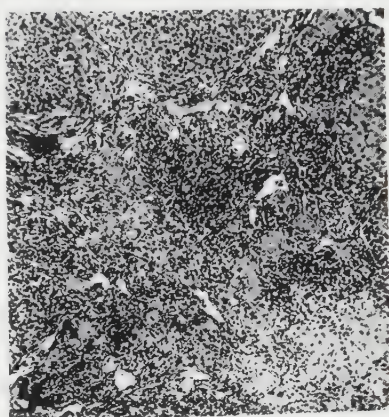
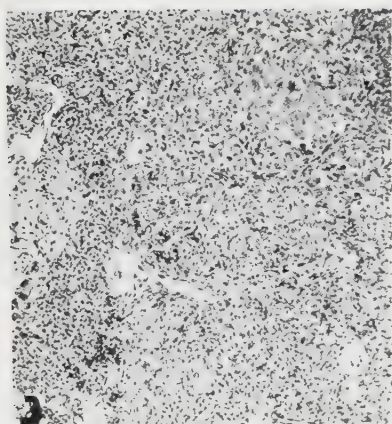


PLATE 2

EXPLANATION OF FIGURES

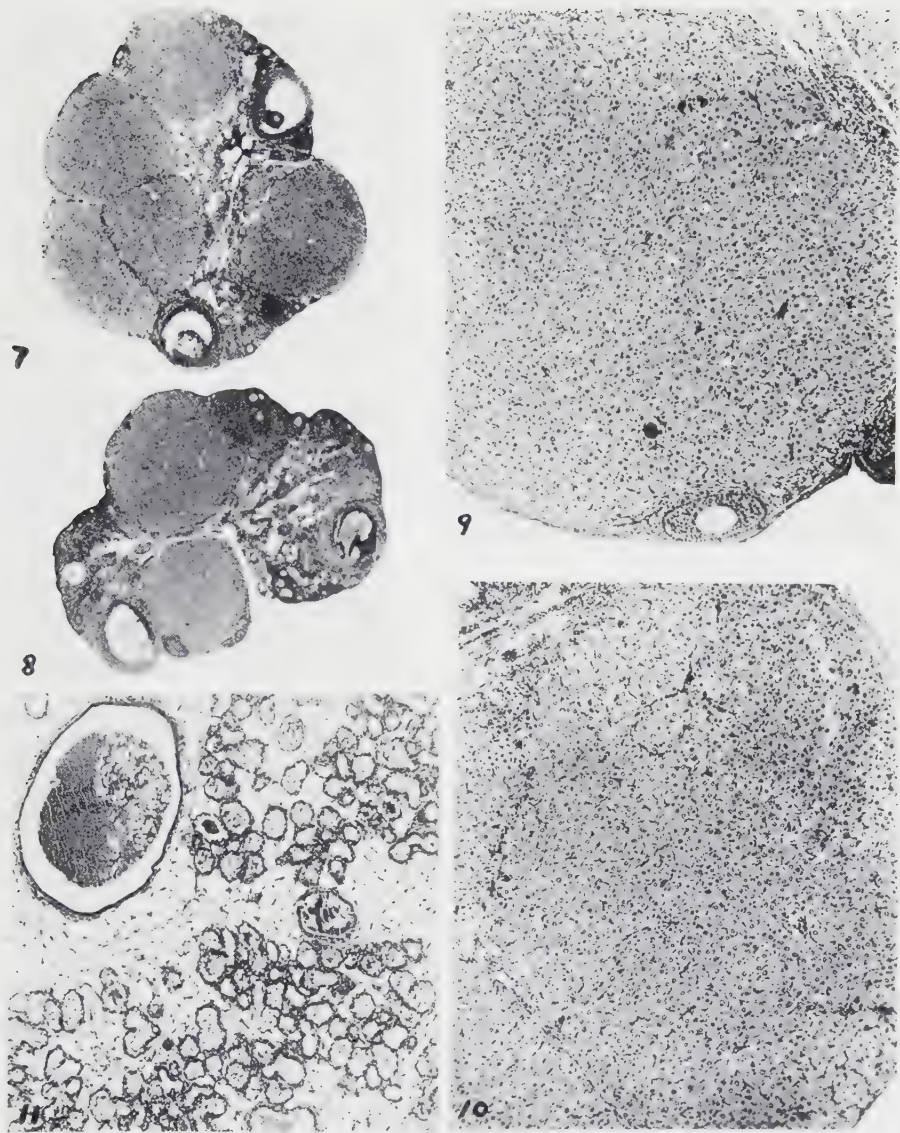
7 Ovary of an untreated pregnant mouse showing four corpora lutea of gestation.

8 Ovary of a progesterone treated pregnant mouse with two corpora lutea of approximately the same size as those seen in the ovary of the untreated pregnant control shown in figure 7.

9 Normal corpus luteum of gestation in the ovary of an untreated mouse.

10 Normal corpus luteum of gestation in the ovary of a progesterone treated mouse. Its structure is the same as that of the untreated pregnant control shown in figure 9.

11 Fully lactating mammary gland of a progesterone treated rat on the day of delivery. It will be noted that the galactophores and acini are full of milk in spite of the treatment.



EFFECT OF SEX HORMONES ON THE GONADS OF FROG LARVAE (*RANA CLAMITANS*): SEX INVERSION IN FEMALES; STABILITY IN MALES¹

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THREE PLATES (SEVEN FIGURES)

Among the vertebrates there is no group of animals which have more often or more successfully served in experiments on sex reversal than the frogs. Even before fertilization, by allowing the eggs to become 'overripe' through prolonged retention in the uteri, a distinct change of the sex ratio in favor of the males was obtained. Low temperatures applied to larvae at the stage of sex differentiation shift the ratio temporarily in the direction of the female, while high temperatures at the same stage or even after completion of normal sex differentiation cause female larvae to transform into males. After the discovery of the sex hormones it was only natural that their possible effect on sex differentiation in tadpoles should be tested. In 1930 we injected estrogenic hormones extracted from urine of pregnant women into the larvae of *Rana clamitans*. Although fairly large quantities were administered no effect beyond unspecific degeneration in the ovaries could be detected. However, in 1936 there appeared a paper by Padoa announcing a 'paradoxical' masculinizing effect of an ovarian extract 'Cristallovar.' First reports on the effect of pure androgens appeared a year later when it became evident that testosterone very definitely causes a transformation of genetical females into males (Gallien, '37, '38; Witschi and Crown, '37; Foote, '38; for

¹ Aided by grants from the National Research Council, Committee for Research in Problems of Sex. The authors are also indebted to the Ciba Pharmaceutical Products, Inc. for the generous supply of sex hormones.

review and discussion of this literature see Witschi, '39). It also seems probable that estrogens will delay or entirely suppress testicular differentiation, at least in larvae of the undifferentiated frog races.

The experiments mentioned above were mostly performed with larvae not yet sexually differentiated at the start of treatments and of unknown sex constitution. In the fall of 1938 we decided to make a series of experiments with older larvae of *Rana clamitans*, which were long past the stage of sex differentiation (figs. 1 to 4). In this species metamorphosis occurs relatively late, at about the age of 1 year. In the fall the larvae are quite large with bodies of the shape and size of pigeon or small hen's eggs. Their sex can easily be ascertained by exploratory laparotomy. By this means it was possible to segregate 124 males and 138 females. They were divided into several groups and treated as indicated in table 1. The large size of the larvae and their roomy body cavities suggest at once the method of intraperitoneal injection for the administration of hormones. The hormones were dissolved in oil which prevents their rapid elimination through the open nephrostomes. Oil apparently does not pass out through them but remains for a long period of time in the body cavity, becoming enclosed in a meshwork of mesenchyme which closely resembles the structure of the normal fat bodies. Injections were made once a week or biweekly.

The injection of 270 γ of Estradiol and Estradiol 3-benzoate 17-butyrate (about 4000 R.U.) over a period of 4½ months has little effect on the testes. Neither the seminal tubules nor the spermatogonia seem much affected. However, the tubules of the rete testis show a tendency to become somewhat inflated, a development which reminds one of the fact that the rete of the testis is the homologue of the ovarian sacs of the ovary. As a whole these experiments are as negative as those of 1930, though now there is less degeneration, due to the greater purity of the new hormone preparations.

Most spectacular is the effect which the testosterone (Perandren Ciba) injections produce in the ovaries (table 2).

TABLE 1

Controls and experimental larvae of Rana clamitans: 138 females + 124 males

NUMBER AND SEX	TREATMENT	OBSERVED EFFECTS
32 ♂	Estradiol 3-benzoate 17-butyrate followed by Estradiol	Minor modifications in testicular structure
4 ♂	Solvent (corn oil, Mazola)	} Controls killed at end of experi- ment; normal testes
4 ♂		
1 ♂	Testosterone propionate	None
19 ♂	Estradiol	Still alive
20 ♂	Estradiol + gonadotropin	Experiment not yet terminated
38 ♀	Testosterone propionate	Progressive sex inversion (table 2)
6 ♀	Solvent (corn oil, Mazola)	} Controls killed at end of experi- ment; normal ovaries
5 ♀		
17 ♀	Testosterone	Still alive
23 ♀	Testosterone propionate + gonadotropin	Experiment not yet terminated
44 ♂		} Controls killed at start of experi- ment
49 ♀		

TABLE 2

*Progressive sex inversion of thirty-eight female larvae treated with
testosterone propionate*

DAYS AFTER FIRST INJECTION	AMOUNT INJECTED PER LARVA γ	NUMBER OF ANIMALS AND TYPE OF REACTION				Almost typical testes
		Ovocyte degeneration	Theca proliferation	Organization of male rete	Sex cord formation	
15	500		2			
23	500	1	1			
29	750	2				
37	1000			2		
51	1250		1	1		
67	1500			2		
81	1750		1			
95	2000			1	1	
133	3250			3	5	7
137	3500				1	7
Totals		3	5	9	7	14

As early as 1 month after start of the treatments the ovaries begin to shrink. After the fourth month they appear testis-like, though they are still somewhat larger and more irregular in shape than normal male gonads. On cross sections (figs. 3 to 7) one observes a rapid degeneration of the ovocytes. The youngest ones disappear first; the larger, yolk laden ones are more resistant (figs. 6 and 7), some fragments being still present at the end of the fourth month (fig. 5). The disappearance of the ovocytes is accompanied by the proliferation of the walls of the ovarian sacs (which form the thecae of the egg follicles), the development of a rete testis with vasa efferentia and the formation of sex cords. The latter are of a particular interest. The only germ cells which do not suffer degeneration are the primary ovogonia. They become included in the sex cords and carried to the medullary parts of the glands, where they assume the value of primary spermatogonia.

The rapid and radical transformation of the ovaries is the more surprising as it occurs at a time when the normal larvae change very little, grow slowly if at all, and the gonads seem to be completely at rest. Very few of the tadpoles metamorphose during the course of the winter.

Here as in all previous experiments on sex differentiation the question arises whether the experimental factors—this time the sex hormones—directly replace the normally acting inductors, or merely shift their equilibrium, that is, the genetically established ratio between cortex and medulla. A final answer cannot yet be rendered. The morphological changes in the testosterone induced transformation of ovaries into testes conform very closely with those observed in the normal reversal of ovaries into testes in frogs of undifferentiated race (Witschi, '29); therefore either of the two interpretations is equally well applicable. On the other hand, present and previous experiments with gynogenic substances indicate that in the male a shift toward ovarian development is possible only as long as some cortex is left. This is distinctly in favor of the alternative that the hormones (like

low temperatures in earlier experiments) act indirectly, by favoring cortical or suppressing medullary induction.

The combined effects of sex hormones and gonadotropins will be described in a forthcoming paper.

SUMMARY

In sexually differentiated larvae of *Rana clamitans* transformation of ovaries into testes is induced by testosterone propionate. On the other hand, estrogenic hormones have little effect on the structure of the testes. The observed facts suggest an indirect action of the sex hormones in all cases where the normal course of differentiation of the gonads becomes modified.

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PLATE 1

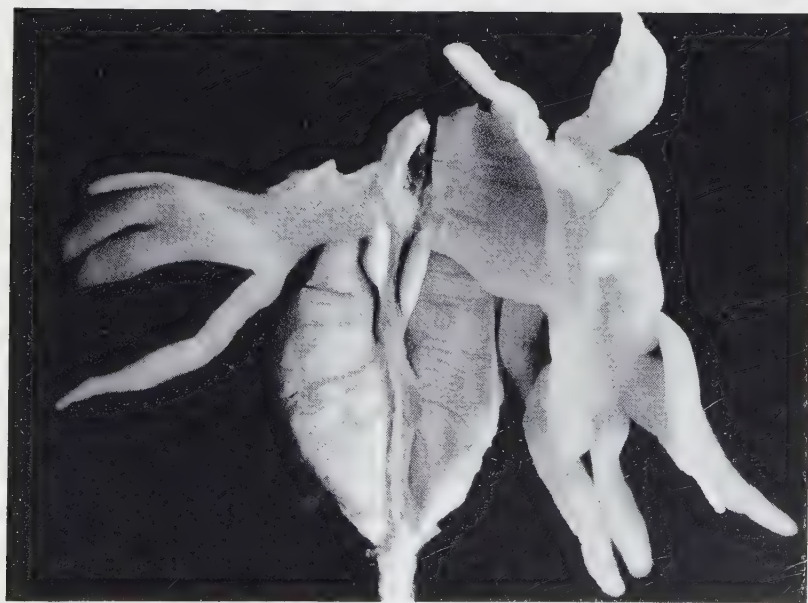
EXPLANATION OF FIGURES

1 Urogenital organs of a female larva of *Rana clamitans* of 60 mm. total length (body 28 mm.). The tip of the right mesonephric body has been cut off. Characteristic form of the large fat bodies and folded, ribbon-like ovaries of the winter season. Oviducts not yet formed. $\times 6$

2 Urogenital organs of a male larva of 50 mm. total length. Note the relatively small size and smooth surface of the testes, characteristic of the winter season. $\times 6$.



1



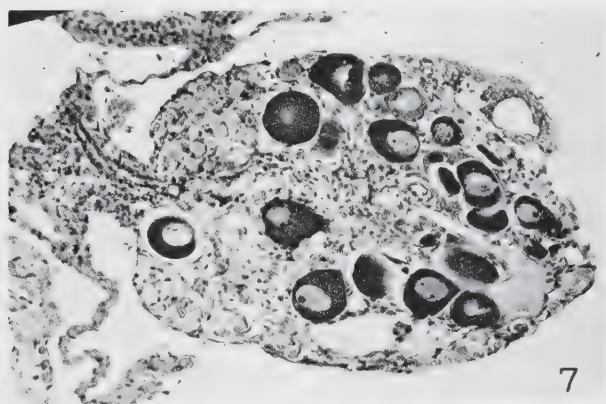
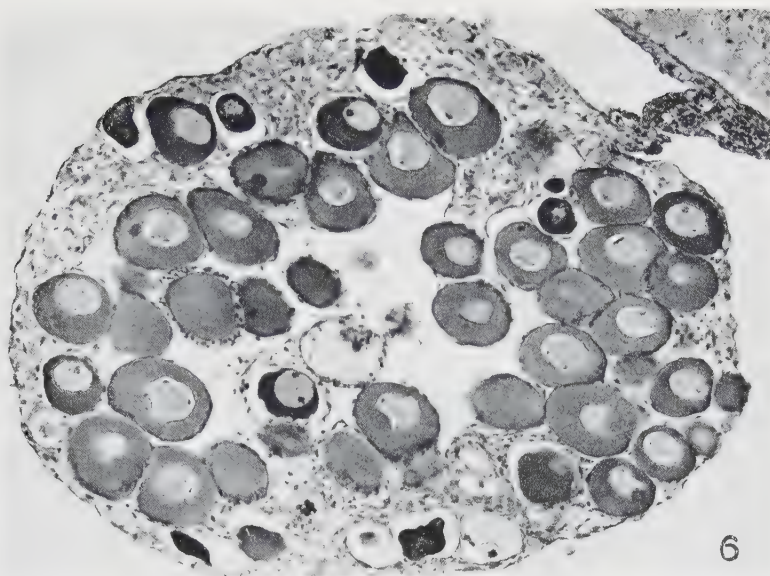
2



3 Cross section through ovary of a control larva preserved at the end of the experiments. $\times 100$.

4 Cross section through testis of a control larva preserved at the end of the experiments. $\times 100$.

5 Cross section through the testis of a sex-reversed female larva, after $4\frac{1}{2}$ months of treatment with testosterone propionate (total 3550γ); note the remains of a degenerated egg near the center. $\times 100$.



6 Cross section through gonad of a female larva after 95 days of treatment with testosterone propionate (total 2000 γ); note degeneration of the ovocytes. $\times 100$.

7 Cross section through gonad of a female larva after 133 days of treatment with testosterone propionate (total 3250 γ); note proliferation in the medullary region and the formation of sex cords and vasa efferentia. $\times 100$.

ACTION OF ESTRONE ON SEXUAL ORGANS OF IMMATURE MALE CATS

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ONE PLATE (EIGHT FIGURES)

Although numerous investigators have considered the effect of estrogenic compounds on the male secondary sexual organs, there is still lack of agreement as to the effect of these compounds on the prostate gland. Thus while earlier studies on mice have demonstrated that estrone causes hypertrophy of the prostate and metaplasia of its epithelium (Lacassagne, '33; Burrows and Kennaway, '34), later studies on rats and guinea pigs have shown that atrophy of the organ occurs when the treatment is prolonged even although shorter treatment with higher dosages induces hypertrophy (Noble, '38; del Castillo and Pinto, '38). In the monkey, Parkes and Zuckerman ('35) and Courrier and Gros ('35) have reported an increase in the collagenous tissue of the prostate with a reduction in size and number of the prostatic glands following estrone treatment, but in the dog, injection of these compounds is followed by an increase in growth of the fibromuscular tissue and an epithelial hyperplasia of the organ.

Since species differences seem to play such a role the present investigation was undertaken to study the reaction of the prostate and testis in the immature male cat. While this work was in progress, Courrier and Gros ('38) reported that prostatic hypertrophy could be induced in normal 20-day-old cats and in castrate animals receiving 0.2 mg. of estradiol daily for 20 days. This hypertrophy was due to a stimulation of the glandular epithelium. Our experiments extend

the study to include varied ages of immature animals and varied modes of treatment.

Seventeen immature male cats ranging in age from 5 to 12 weeks were used for these experiments. The age of each animal was definitely known. Seven animals served as controls, of which six were litter mates in the group in which they were employed.

Progynon-B,¹ in sesame oil, was injected intramuscularly in 100 rat unit dosages for periods of 10, 15, 20, 25, and 30 days. Two cats received 200 rat units daily for 15 days. All animals were sacrificed 24 hours after the last injection. The animals did not exhibit any deleterious effects as the result of the injections.

Weights of the testis did not show marked differences after estrone treatment. In general, where litter-mate controls were available, the weight of the testes was the same or slightly lower in the injected group although the body weights were quite comparable. It was of interest to note that the two cats that received 3000 R.U. of estrone in 15 days had testes which were 38% and 50% heavier than the control weights. However, no definite conclusions as to weight variations could be drawn from this small number of experiments.

Histologically the testes showed virtually no influence as the result of treatment. The animals treated for 20 and 30 days showed a slight indication of atrophy. A possible increase in spermatogenesis as the result of high dosage with short treatment duration was suggested by the results in rats and guinea pigs as reported by del Castillo and Pinto ('38), but no evidence of stimulation was observed in the two cats receiving 3000 R.U. in 15 days.

True squamous metaplasia in the urethral epithelium has been observed in cats by Raynaud ('37). In our experiments, under varied duration of treatment, metaplasia of the urethral epithelium was observed in all animals and to virtually the same degree. Whereas the epithelium of the immature con-

¹ The Progynon-B used in this investigation was supplied by the Schering Corporation through the kindness of Dr. Max Gilbert.

trol (figs. 1 and 2) was only two or three cells in thickness, the treated animals showed as much as a fivefold increase (figs. 3 and 4) with a desquamation of layers of superficial cells in some instances. Squamous metaplasia of the ductal epithelium also resulted. Leucocytes were rare in either the connective tissue or the epithelium and mitotic figures were very sparse.

Weights of the prostates were not taken to ascertain the degree of hypertrophy, but the histology was considered in all cases. The immature control animals showed prostatic alveoli with small lumina and with cuboidal or low columnar epithelium (figs. 5 and 6). After 15 days treatment with 100 R.U. doses of estrone, the prostatic glands increased, the lumina were enlarged, and the epithelium was columnar. After 25 days, the glandular epithelium was hypertrophied to a tall columnar type (figs. 7 and 8). The longer treatment was more effective than a fairly comparable dosage administered over a shorter interval.

Previous investigators, Courrier and Gross ('38), observed stimulation of the glandular epithelium at 20 days. We have observed definite stimulation at 15 days, but no atrophy was evident at 25 to 30 days, under the conditions of these experiments.

SUMMARY

Hypertrophy of the glandular epithelium of the prostate resulted in immature male cats treated with estrone. Furthermore, metaplasia of the urethral and ductal epithelium also occurred in the absence of leucocytic infiltration.

Acknowledgment is gratefully made to Dr. J. C. Donaldson for his criticisms and suggestions in connections with this work.

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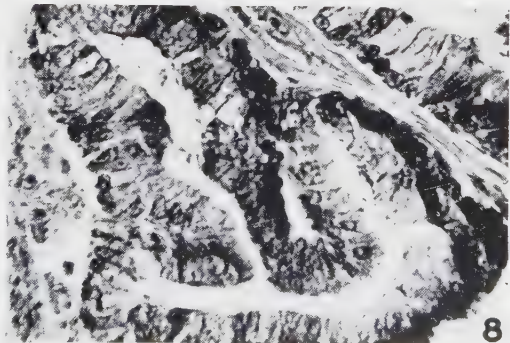
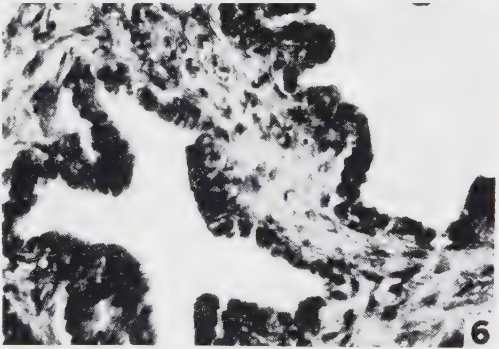
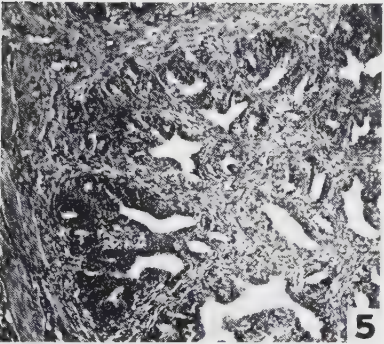
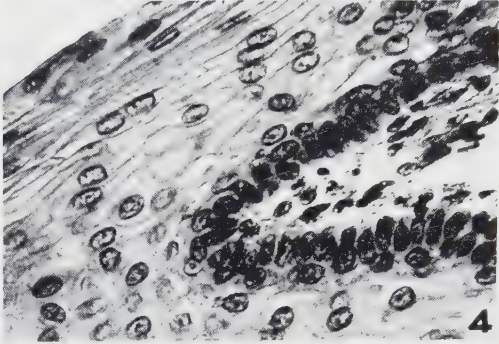
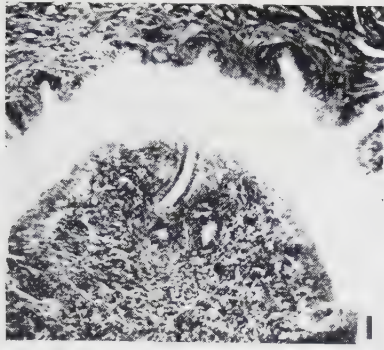
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PLATE 1

EXPLANATION OF FIGURES

- 1 Urethra of normal untreated immature male cat. $\times 85$.
- 2 A representative section of control urethra as shown in figure 1. $\times 371$.
- 3 Urethra of immature male cat treated with 100 R.U. of Progynon B daily for 25 days. Note the extent of squamous metaplasia. $\times 85$.
- 4 A representative section as shown in figure 3 indicating the detail of the urethral squamous metaplasia. $\times 371$.
- 5 Prostate of normal untreated male cat. $\times 85$.
- 6 A representative section of normal prostate as shown in figure 5. Note the height of the epithelial lining of the prostatic glands. $\times 371$.
- 7 Prostate of immature male cat treated with 100 R.U. of Progynon-B daily for 25 days. Note increase in number and size of prostatic glands. $\times 85$.
- 8 A representative area from figure 7. Note the experimentally induced hyperplasia of the prostatic glandular epithelium. $\times 371$.



THE EFFECTS OF CONTINUOUS INTRAVENOUS IN-
JECTION OF DEXTROSE IN INCREASING
AMOUNTS ON THE BLOOD SUGAR
LEVEL, PANCREATIC ISLANDS
AND LIVER OF GUINEA PIGS

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ONE PLATE (THREE FIGURES)

In an earlier paper, the effects of continuous intravenous injection into guinea pigs of approximately 1 gm. of dextrose per kilogram of body weight per hour for varying periods of time were described (Woerner, '38). These studies have been extended with the administration of 2 to 3 plus grams of dextrose per kilogram of body weight per hour for varying periods of time. In this series twenty male guinea pigs were used; nine animals injected with 2 plus grams per kilo per hour, eleven animals with 3 plus grams.

The method of continuous intravenous injection was the same as that described in the earlier series with the exceptions that the sugar was preceded by a recovery period of 24 hours, during which time normal saline solution was injected at the rate of 2 cc. per hour, a 50% solution of glucose was used in some cases instead of a 30% solution and in some cases the rate was increased from 2 to 4 cc. per hour. The methods for obtaining tissue for cytological studies were the same as in the earlier series. By employing the micro-method of Miller and Van Slyke ('36) for quantitative blood and urine sugar analyses instead of the Somygi modification of the Shaffer-Hartman method, many more determinations could be made during the course of an experiment.

Animals receiving 3 plus grams per kilo per hour continuously did not often survive much longer than 2 days. For this reason the injection was interrupted for 24 hours in the case of one animal (no. 68), in order to study the effects of more prolonged injection.

Following are given the protocols of selected animals from each group where the tissue was adequate for cytological studies and the sugar analyses were successfully made. The physiological description of the alpha and beta cells is made on the basis of the correlation of cytological changes with functional states of the cell described in an earlier paper (Bensley and Woerner, '38).

GROUP A. ANIMALS RECEIVING 2 TO 2 PLUS GRAMS OF DEXTROSE PER KILOGRAM OF BODY WEIGHT PER HOUR

I. *Animal no. 78. Twenty hours of 2.0 gm.*

<i>Time</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
20 hours	800 plus	8.0
		(bladder urine—8.3%)

1. Pancreas:

Beta cells.

- 1) Many almost agranular, some with rod-like mitochondria, suggesting active exhaustion; a few with globular mitochondria, suggesting beginning degenerative changes.
- 2) Some have polar distribution of granules. Most of these have rod-like mitochondria, suggesting active secretion. Some have granular mitochondria.
- 3) A few are filled with granules, their mitochondria being oval to globular, suggesting secretory inactivity. No evidences of increase in the number by mitosis or transformation were found.

Alpha cells.

- 1) The majority are well filled with granules and contain tiny vacuoles, their mitochondria not visualized. Apparently these cells were not actively secreting.
- 2) A few have polar distribution of the granules and contain rod-like mitochondria, suggestive of actively secreting cells.
- 3) A few are agranular and shrunken, their mitochondria not visualized, suggesting atrophic cells.

2. Liver:

Cells well filled with glycogen, contain small round vacuoles but no osmic combining fat.

II. *Animal no. 59.* Four days of 2.44 gm. (buffered).

<i>Time day</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
First		2.72
Second		3.29
Third		8.00
Fourth	1670	9.55

1. Pancreas (fig. 1):

Beta cells.

- 1) Many are completely agranular. In most of these the mitochondria are globular, suggesting degenerative changes. In some the mitochondria are rod-like. A few mitoses are found in agranular cells with rod-like mitochondria.
- 2) Some contain polar distribution of granules with rod-like mitochondria, suggesting secretory activity.
- 3) Rarely a cell filled with granules, containing globular mitochondria, suggesting secretory inactivity.

Alpha cells.

Almost all are well filled with granules (their mitochondria not visualized). Apparently these cells were not actively secreting.

2. Liver:

Cells are distended with glycogen and contain a moderate amount of osmic combining fat. Almost all Kupffer cells contain some fat.

III. *Animal no. 62.* Eight days of 2.0 gm. (buffered).

<i>Time</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
Before injection	120	
After 5 hours	220	1.9
After 1 day	253	2.0
After 2 days	248-258	2.0
After 3 days		2.75
After 4 days	167	
After 8 days	247	0.30

1. Pancreas (fig. 2):

Beta cells.

- 1) Many have polar distribution of granules. Most of these have mitochondria which are long large rods. A few have globular mitochondria.
 - 2) Quite a few are agranular; some with rod-like and some with globular mitochondria.
 - 3) A few filled with granules, some containing rod-like mitochondria, some with globular mitochondria.
- Many evidences of increase in number by mitosis and transformation were found.

Alpha cells

Very few typical alpha cells to be found.

1) Some almost agranular with rod-like mitochondria.

2) Some with polar distribution of granules with rod-like mitochondria.

One has the impression that there are more undifferentiated cells than are usually found in the islands.

2. Liver:

Cells moderately enlarged and filled with glycogen. Very little osmic combining fat in liver cells. Kupffer cells contain some small black droplets.

GROUP B. ANIMALS RECEIVING 3 TO 3 PLUS GRAMS OF DEXTROSE
PER KILOGRAM OF BODY WEIGHT PER HOUR

I. *Animal no. 56.* Forty-eight hours of 3.2 gm.

<i>Time</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
Before injection	78	
After 22 hours	118	
After 2 days	213	2.54

1. Pancreas:

Beta cells.

1) Many have polar distribution of granules. Most of these contain rod-like mitochondria, some contain globular mitochondria.

2) A few filled with granules and contain globular mitochondria.

No evidence of increase in number by mitosis or transformation.

Alpha cells

1) The majority are well-filled with granules and contain mitochondria that are tiny rods and granules.

2) Some with polar distribution of granules containing rod-like mitochondria.

2. Liver:

Cells large and well filled with glycogen. Large droplets of osmic combining fat occur toward the central vein; clusters of small black droplets are found at the periphery of the lobule.

II. *Animal no. 60.*

1 day of 1.1 grams

1 day of 2.2 grams

1 day of 3.3 grams

<i>Time</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
After second day	260	
After third day	1316-1348	9.42

1. Pancreas:

Beta cells.

- 1) Many with polar distribution of granules with mitochondria varying from rods to globules.
- 2) Some filled with granules and containing globular mitochondria.
- 3) A few agranular with globular mitochondria.

Alpha cells

The majority are well filled with granules, containing tiny vacuoles and thick rod-like mitochondria.

2. Liver:

Cells large and filled with glycogen. Not much fat in the liver. A few cells contain rather large osmic combining fat globules.

III. *Animal no. 52.*

9 days of 1.2 gm.
42 hours of 3.6 gm.

<i>Time</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
Ninth day	190	
90 minutes of 3.6	280-320	
13 hours of 3.6	560-720 (640)	
18 hours of 3.6	440-460	
24 hours of 3.6		3.44
36 hours of 3.6	840-860	
42 hours of 3.6	1260-1300	4.70

1. Pancreas:

Beta cells.

- 1) Some with polar distribution of granules. Some of these contain rod-like mitochondria, more contain globular mitochondria.
 - 2) Some agranular with globular mitochondria.
 - 3) Some filled with granules and with globular mitochondria.
- Some evidence of increase in number by mitosis.

Alpha cells

The majority are well filled with granules, contain tiny vacuoles and filamentous mitochondria.

2. Liver:

Cells large and well filled with glycogen. Large osmic-combining fat globules occur in cells toward the central veins.

IV. *Animal no. 68.*

	2 days of 3.17 grams	
	1 day of rest	
	2 days of 3.17 grams	
<i>Time</i>	<i>Blood sugar mg./100 cc.</i>	<i>Urine sugar %</i>
Before injection	108	
After 24 hours	121-117	4.8
After 48 hours	363	9.8
Injection stopped		
in 30 minutes	254	
in 60 minutes	188	
in 90 minutes	141	
in 2 hours	138	
After 1 day of rest		0.19
Injection resumed		
After 24 hours		7.74
After 48 hours	595	11.20
Injection stopped		
in 90 minutes	333	
in 2 hours	323	

1. Pancreas (fig. 3):

Beta cells.

- 1) The majority are agranular. In some the mitochondria are globular, in most they are rod-like.
- 2) Some filled with granules and contain small granular mitochondria.
- 3) Occasional mitoses are found in agranular cells with rod-like mitochondria.

Alpha cells

- 1) The majority are loaded with granules and contain filamentous mitochondria.
- 2) A few have polar distribution of granules with rod-like mitochondria.
- 3) A few shrunken and agranular; the mitochondria not visualized.

2. Liver:

Cells distended with glycogen. Some cells contain masses of fat that partially or completely blacken with osmic acid. Osmic combining fat globules appear to be even in the central vein.

DISCUSSION

From these experiments it is apparent that the degree of hyperglycemia produced by increased amounts of intravenous dextrose is very variable. But the correlation between the degree of hyperglycemia and the condition of the beta cells is more uniform. Where the response of the beta cells to the intravenous dextrose is maximal and there is no evidence of

hyperplasia the level of the blood sugar has been progressively increased. When, however, the secretory activity of the beta cells is accompanied by mitotic activity there appears to be some cyclic evidence of partial adjustment of the blood sugar level. Animal no. 52 is an apparent partial exception to this generalization.

The condition of the alpha cells is in sharp contrast to that found after the injection of 1 gm. of dextrose per kilo per hour. In the latter case the alpha cells were exhausted and in some instances, hydropic. With increasing amounts of sugar they appear to be actively secreting and finally suppressed.

In this series the liver cells are well filled with glycogen in contrast to the liver cells of the animals receiving only 1 gm. per kilo per hour in which case they contained very little glycogen.

The relationship of the secretory activity of the alpha cells to the accumulation of fat in the liver is not as clear. However, it is apparent that when the alpha and beta cells are both actively secreting there is little osmic combining fat to be found in the liver. It also appears that with suppression of secretion of the alpha cells osmic combining fat occurs in the cells chiefly toward the central veins and in the Kupffer cells. But from this series it is not clear which precedes and which follows, or whether they are coincident.

It is interesting to note that in the case of animal no. 68 the blood sugar level obtained after the second 48 hours of injection was 1.6 plus times that of the level after the first 48 hours of injection although the same amount was being injected and there was a recovery period of 24 hours between, during which time glycosuria almost completely disappeared. This would suggest either that during the first 48 hours of intravenous injection some of the exhausted beta cells were damaged and that any proliferative response in the remaining beta cells was not adequate to control the blood sugar in the second period to the extent of that in the first, or that the liver glycogen was not removed during the 24-hour recovery

period, or that liver function was impaired and therefore sugar was not as effectively removed from the blood by this organ.

The appearance of mitoses in the exhausted beta cells may be correlated with the fact that the animal had a 2-hour recovery period, during which time blood sugar determinations were made, before the animal was killed. This would suggest that although the beta cells were exhausted they were not all damaged.

Although most of the alpha cells are loaded with granules the appearance of a few actively secreting cells may be correlated with this premortem period of recovery. The appearance of the fat in the liver is suggestive of its removal and perhaps may be correlated with the activity of some of the alpha cells.

The results of continuous intravenous injection of varying amounts of sugar into guinea pigs are not entirely in accord with those found by Jacobs and Colwell ('36) in dogs. These authors state: ". . . . The continuous administration of glucose even at rates just within the limits of tolerance, that is, not causing glycosuria, is always fatal. . . . higher injection rates, causing glycosuria, are more rapidly fatal. The mechanism leading to death would appear to be a non-ketogenic acidosis associated with profound congestive and hemorrhagic changes, particularly involving the pancreas and anterior hypophysis."

In guinea pigs the administration of dextrose was not always fatal. In over sixty animals examined, frank hemorrhage in the pancreas was never found. Higher injection rates were not always more rapidly fatal.

The results are in accord in that the individual variation of animals to intravenous glucose was considerable, the storage of glycogen was remarkable with the higher rates of injected sugar, and that, in general, greater amounts of glucose were more often fatal.

The differences in the results may be due in part to differences in the character of the experiments. The sugar injected into dogs was dissolved in water whereas that injected

into guinea pigs was dissolved in isotonic salt solution. The dogs were deprived of food whereas the guinea pigs were fed. The concentration of the sugar solution injected was different except in the case of the higher rates. In addition, it may be that compensation in the guinea pig is more readily effected than in the dog.

The data obtained from guinea pigs corroborate the findings of Felsher and Woodyatt ('24) and Jacobs and Colwell ('36) in that the tolerance of normal animals to glucose varies somewhat with the individual animal and is approximately 1 gm. per kilogram of body weight per hour.

CONCLUSIONS

From these experiments it appears that although there is considerable individual variation in the reaction of the islands of different guinea pigs to the amount of dextrose continuously injected intravenously, there is an apparent correlation between the blood sugar level and the condition of the island cells. There is also some evidence that the reaction of the island cells to the sugar in the blood is a phasic or cyclic one.

The results indicate that when the intravenous injection of dextrose is accompanied by considerable hyperplasia and secretory activity of the beta cells and the blood sugar level is raised only slightly above the feeding level the alpha cells may be exhausted and even hydropic, little glycogen may be stored in the liver and no osmic combining fat is found there (Woerner, '38).

When the injection is accompanied by actively secreting and exhausted beta cells with some mitotic activity and the blood sugar level is considerably raised (i.e., two to three times the normal level in 24 to 48 hours), the alpha cells may be actively secreting and somewhat suppressed, the liver cells may be filled with glycogen and very little osmic combining fat found in that organ.

When the blood sugar level is excessively high, the beta cells exhausted and degenerating or suppressed, the alpha

cells may be almost completely suppressed, the liver cells distended with glycogen and increasing amounts of osmic combining fat found in the Kupffer cells and in the liver cells toward the central vein.

The cytological studies suggest that the functional state of the island cells may not be always accurately determined by the granule content of the cells alone. An agranular cell may be either temporarily functionally exhausted, or degenerating either from exhaustion or suppression. Whereas a cell filled with granules may be in the resting state, suppressed, or in the stage of granule formation. Simultaneous studies of the mitochondria and Golgi nets suggest means of differentiating functional phases in the granular and agranular cells. However, more experimental material is needed before these correlations may be made absolute.

SUMMARY

With the continuous intravenous injections of 2 to 3 plus grams of dextrose per kilogram of body weight per hour into guinea pigs for varying periods of time, the following results were observed.

1. The liver cells were filled or distended with glycogen. The distention of the cells with glycogen may have impaired liver function and this may account for the fact that animals injected continuously with 3 plus grams did not often survive much longer than 2 days.

2. The increase in blood sugar level was very variable and in some cases showed fluctuations during the course of the experiment when frequent determinations were made.

3. There appeared to be some correlation between the terminal blood sugar level and the condition of the cells in the islands of Langerhans. Where the blood sugar was high, usually the majority of the beta cells were exhausted or showed beginning degenerative changes and the majority of the alpha cells appeared to be suppressed. Where the blood sugar level was only moderately elevated the majority of

the beta cells and the alpha cells appeared to be actively secreting.

4. There appeared to be some correlation between the liver glycogen and fat and the condition of the island cells. When the majority of both beta and alpha cells appeared to be actively secreting, the liver cells were well filled with glycogen and not much osmic combining fat was found in them. When the majority of beta cells were exhausted or showed beginning degenerative changes, and the alpha cells appeared inactive or suppressed, the liver cells were distended with glycogen and osmic combining fat occurred in the cells toward the central vein and in the Kupffer cells.

I wish to express my gratitude to Drs. R. R. Bensley and S. H. Bensley for their suggestions and interpretations of the cytological material, and to Mr. R. D. Bensley for the photomicrographs.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

The optical system used for figures 1 to 3 consisted of a Zeiss apochromatic oil immersion lens H.I. $\times 35$, 0.85 aperture and a Leitz Homel ocular, with a bellows length which gave a magnification of $\times 645$.

The line drawings were made by tracing outlines in the photomicrographs, the details being checked by microscopic observation of the cells photographed.

1 and 1 a Four-micra section of pancreas of guinea pig no. 59, injected for 4 days with 2.44 gm. of dextrose/kilo/hour, fixed in formalin-chrome-sublimate, stained in neutral gentian. Cells in a large peripheral island.

α , alpha cells; β , beta cells with polar distribution of granules; α - β , agranular beta cells; r, red blood corpuscles.

2 and 2 a Four-micra section of pancreas of guinea pig no. 62, injected for 8 days with 2.0 gm. of dextrose/kilo/hour, fixed in formalin-chrome-sublimate, stained in neutral gentian. Cells in a large interlobular island.

α , alpha cells; α - β , agranular and vacuolated beta cell; ?, undifferentiated cells or modified alpha cells?

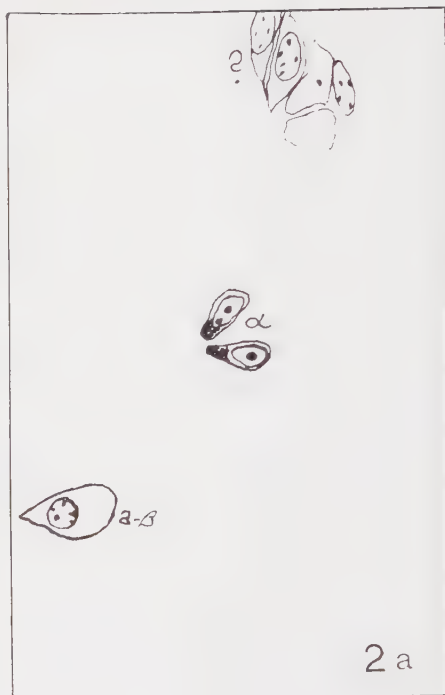
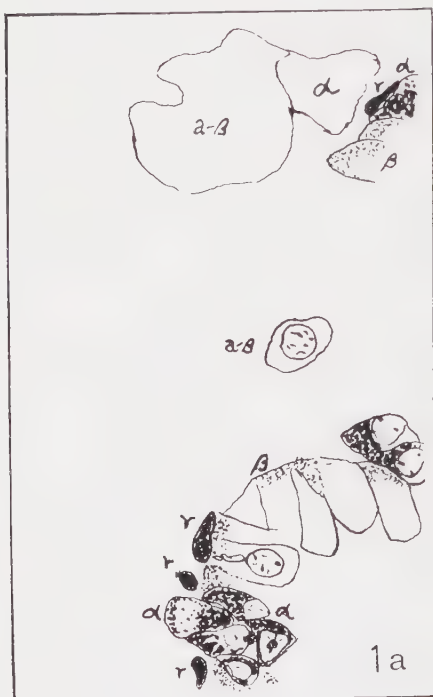
Note the scarcity of typical alpha cells.

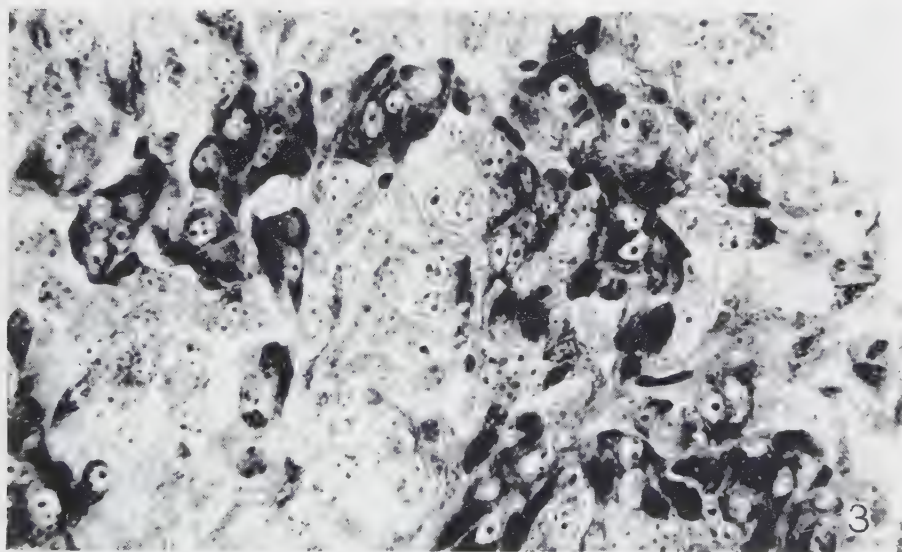
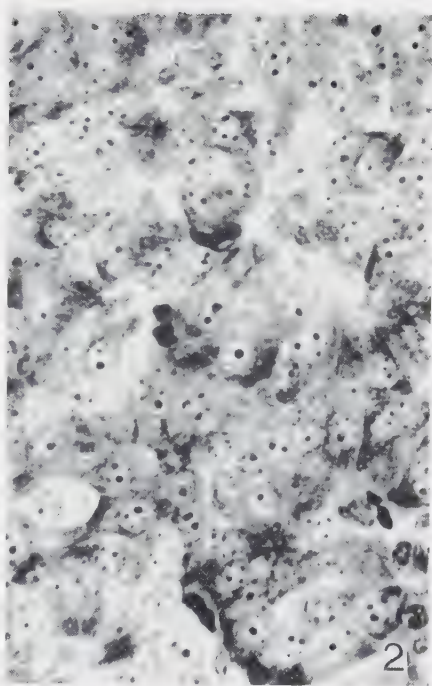
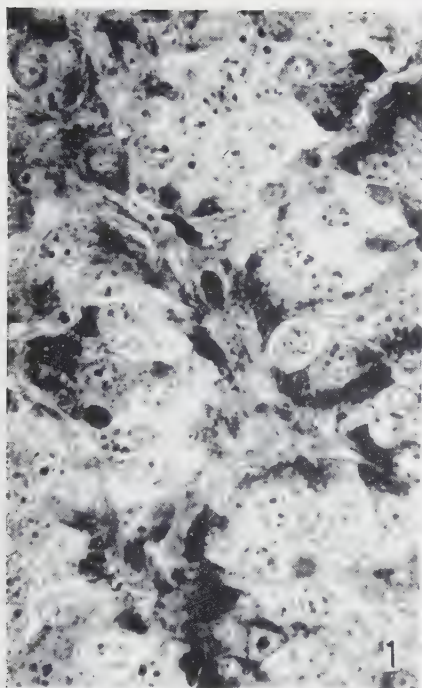
3 and 3 a Four-micra section of pancreas of guinea pig no. 68, injected for 4 days with 3.17 gm. of dextrose/kilo/hour, fixed in formalin-chrome-sublimate, stained in neutral gentian. Cells in a large peripheral island.

All the dark-staining areas are alpha cells loaded with granules except for those regions indicated as β g (beta granules) in figure 3a.

r, red blood corpuscles; v, vacuolated cells.

Note the extensive Golgi nets in the central group of agranular beta cells.





RETROGRADE DEGENERATION OF THE SUPRA- OPTIC NUCLEI AFTER SECTION OF THE INFUNDIBULAR STALK IN THE MONKEY¹

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THREE FIGURES

In a recent monograph, Fisher, Ingram and Ranson ('38) have presented evidence showing that the normal structure and function of the neurohypophysis are dependent upon its innervation via the infundibular stalk by a large tract of unmyelinated nerve fibers whose most important component arises from the supraoptic nuclei of the hypothalamus. In the cat, interruption of these fibers by appropriately placed lesions either in the hypothalamus or in the neurohypophysis results in marked retrograde degeneration of the supraoptic nuclei. In the monkey the same degeneration of the supraoptic nuclei occurs after hypothalamic lesions interrupting the supraoptico-hypophyseal tract.

Recent investigations by Mahoney and Sheehan ('36), Gagel and Mahoney ('36), and Foerster, Gagel and Mahoney ('37) have led these authors to conclude, however, that in the monkey connections between the supraoptic nuclei and the hypophysis are very poorly developed, and presumably, therefore, are of little functional significance.

In the present study some quantitative data on the supraoptico-hypophyseal connections in the monkey have been obtained by counting the cells of the supraoptic nucleus in the normal animal and in animals in which supraoptico-hypophy-

¹Aided by grants from the Rockefeller Foundation and the Committee on Research in Endocrinology of the National Research Council.

seal connections were interrupted by sections of the infundibular stalk and time allowed for retrograde degeneration.

The authors are indebted to Miss Mary Ranson for the preparation of the drawings and photographs.

METHODS

Sections of the infundibular stalk were made through a subtemporal operative approach in a field bordered by the optic and oculomotor nerves and traversed by the internal carotid artery. A tiny knife with a ball tip and a rounded anterior edge was inserted behind the internal carotid artery, retracting it anteriorly and bringing the cutting edge of the knife in front of the infundibular stalk, which was then transected by a backward movement of the knife.

Nine to twelve weeks after operation the animals (*Macaca mulatta*) were killed by bleeding under ether or nembutal anesthesia and the vessels of the head were washed out with normal salt solution and perfused with 10% formalin, so that the brain and hypophysis were fixed in situ. The base of the cranium was carefully dissected away leaving the dura and all the intra-dural contents, including those of the sella, intact. In each case a single block including the hypothalamus and the contents of the sella, together with the dura enclosing them, was removed, embedded in paraffin, and sectioned serially at $10\ \mu$, in the transverse plane. Every fourth section through the series was stained with cresyl violet and every twentieth section with Bodian's silver method. Alternate fourth sections through the region of transection were stained by van Gieson's method.

Mid-sagittal reconstructions of the brain and hypophysis were made with a projection apparatus on millimeter ruled graph paper at a magnification of twenty times and were checked with the microscope. In counting the cells of the supraoptic nucleus, the strip method (Davenport and Barnes, '35) was employed at a magnification of 370 times, and the number of nucleoli in $10\ \mu$ sections at intervals of 80 or $100\ \mu$

through the nucleus was counted and multiplied by eight or ten respectively.

Eleven operated and three normal monkeys, whose brains were prepared for microscopical examination in the same manner, were studied.

LOCATION OF THE LESIONS

The infundibular stalk in the monkey is made up of an enlarged proximal portion called the median eminence, which tapers distally into an elongate infundibular stem. These structures lie between the hypothalamus and the infundibular process (*pars nervosa* or neural lobe), and make up a proportion of the bulk of the neurohypophysis which is by no means negligible.

In six animals the lesions consisted of complete sections of the infundibular stem just below the median eminence, leaving the median eminence and hypothalamus uninjured. The location of the section in a representative animal of this group is shown in figure 1A.

In three animals the sections were made at a more proximal level and the median eminence was completely transected. The location of the section in one of these animals is shown in figure 1B, and no injury of the hypothalamus occurred in this case. In the second animal a comparable section resulted in very slight injury to the base of the tuber immediately adjacent to the attachment of the median eminence. In the third animal a section at about the same level involved the vascular supply to the tuberal region of the hypothalamus on the side opposite that approached and a strictly unilateral softening of the tuber occurred. The supraoptic nucleus on the side of the hypothalamus which was uninjured was studied.

In one animal, only the anterior or ventral half of the median eminence was sectioned, as shown in figure 1C. The hypothalamus was entirely uninjured.

In another animal the posterior or dorsal half alone of the median eminence was sectioned as shown in figure 1D. In this



Fig. 1 Midsagittal reconstructions of the hypothalamus and hypophysis of four operated monkeys, with the lesions shown in solid black. A, transection of the infundibular stem; B, transection of the median eminence; C, section of the anterior half of the median eminence; D, section of the posterior half of the median eminence. Abbreviations are as follows: Hy, hypothalamus; IP, infundibular process; IS, infundibular stem; M, mammillary region; ME, median eminence; MI, massa intermedia of thalamus; OC, optic chiasma; PD, pars distalis; PI, pars intermedia; 3 V, third ventricle.

case the internal carotid artery appeared at operation to be placed unusually far caudally and an unsuccessful attempt was made to reach the stalk rostral to it. This was subsequently found to have produced a softening of the lateral aspect of the optic chiasma which extended dorsally into the medial portion of the supraoptic nucleus on the side of approach. The supraoptic nucleus on the side of the hypothalamus which was uninjured was studied.

Data on the regulation of water exchange and the neurohypophysis in the series of animals from which these cases were taken will be presented elsewhere (Magoun, Fisher and Ranson, '39).

SUPRAOPTIC NUCLEUS

The main body of the supraoptic nucleus in the monkey lies on the lateral aspect of the optic chiasma, anterior to the commencement of the optic tract (Grünthal, '31; Crouch, '34; Papez and Aronson, '34). Though some cells of the nucleus are round, most are ovoid shaped. The interior of the neuron is usually faintly stained and most of the deeply staining Nissl substance is distributed in the periphery. The single nucleus is large and usually slightly ovoid with its long axis parallel to that of the cell in which it is eccentrically placed being nearer one pole than the other and usually nearer one side than the other. The nucleus is clear except for slight chromatin material and for a single prominent nucleolus. In the normal brain the cells are densely packed and form one of the most prominent collections of neurons in the hypothalamus.

After chronic section of the supraoptico-hypophyseal tract a striking loss of cells occurs in the nucleus, the remaining cells are scattered, and the borders of the nucleus are no longer clear-cut. In order to provide some quantitative data on the amount of degeneration, cell counts of the supraoptic nucleus were made in normal monkeys and in animals with transections either through the median eminence or through the infundibular stalk below it. In making these counts three

problems arose with reference to the normal material and these may be considered first.

The first concerns the question of multinucleate cells being present and augmenting the cell counts which were based on the number of nucleoli. Scharrer and Gaupp ('33) and Gaupp, Jr. and Scharrer ('35) have reported numerous multinucleate cells in the supraoptic nucleus of man, but do not refer to such cells in the capuchin monkey and state that they are found much less frequently in animals than in man. In a normal supraoptic nucleus of this series in which over 3000 cells were examined, only two were found which might be interpreted as being binucleate and no polynucleate cells were found. Comparable observations were made in other instances, and multinucleate cells may be considered so extremely rare in the supraoptic nucleus of the macaque as to be negligible in affecting cell counts based on the number of nucleoli.

Secondly, it should be noted that all counts refer to the number of cells in the main body of the supraoptic nucleus, but from the main body of the nucleus a number of typical supraoptic cells arch singly or in groups caudally and medially over the optic tract and extend almost to the midline at the caudal aspect of the optic chiasma, where they form a small but prominent collection of neurons. These cells, which are distributed along the intrahypothalamic course of the supraoptico-hypophyseal tract, were not regarded as a part of the supraoptic nucleus by Grünthal ('31), who designated them as nucleus 7. The present study demonstrates that their inclusion as a part of the supraoptic nucleus by Crouch ('34) and Papez and Aronson ('34) is correct, for they are greatly reduced in number after section of the infundibular stalk. Estimates of the number of these cells on one side of the normal monkey brain range between 3000 and 5000. The scattered and irregular distribution of the cells renders their counting so difficult, however, that they have been purposefully omitted from the counts of the main body of the supraoptic nucleus in this study. In the monkey there is no bridge

of cells connecting the supraoptic and paraventricular nuclei, such as has been described for other forms.

The third point concerns the presence in the marginal dorsal and medial portions of the supraoptic nucleus, and also irregularly through more central parts, of a number of cells which are smaller than their fellows. These small cells of which there are about 2000 to 4000 in the normal brain, have been included in counts of the supraoptic nucleus.

The results of cell counts of the main body of the supraoptic nucleus in the normal monkey and after section of the supraoptico-hypophyseal tract in the infundibular stem or median eminence are presented in table 1. The figures are

TABLE 1

Cell counts of the main body of the supraoptic nucleus on one side of the monkey brain

<i>Normal</i>	<i>Section of infundibular stem</i>		<i>Section of median eminence</i>		<i>Section of anterior half of median eminence</i>	<i>Section of posterior half of median eminence</i>
	Total	Normal appearing	Total	Normal appearing		
31,456	8,832	5,096	5,464	1,952		
34,210	8,976	5,720	6,200	3,240	6,960	26,810
37,160	9,336	5,744	6,432	4,496		
	10,304	6,840				
	10,784	6,320				
	11,640	8,216				
Av. 34,275	9,978	6,322	6,032	3,229	6,960	26,810

given in table 1 just as they were obtained, but because the last two digits are of little significance, throughout the text the counts have been expressed in round numbers. It should be noted that all counts refer to the supraoptic nucleus on one side of the brain.

In three normal monkeys the main body of the supraoptic nucleus was found to contain between 31,000 and 37,000 cells, with an average figure of 34,000 (table 1, column 1). Low and medium power views of a section through the densest portion of the supraoptic nucleus of a normal monkey are presented in figure 2A, and the appearance of the cells is shown in figure 3A.

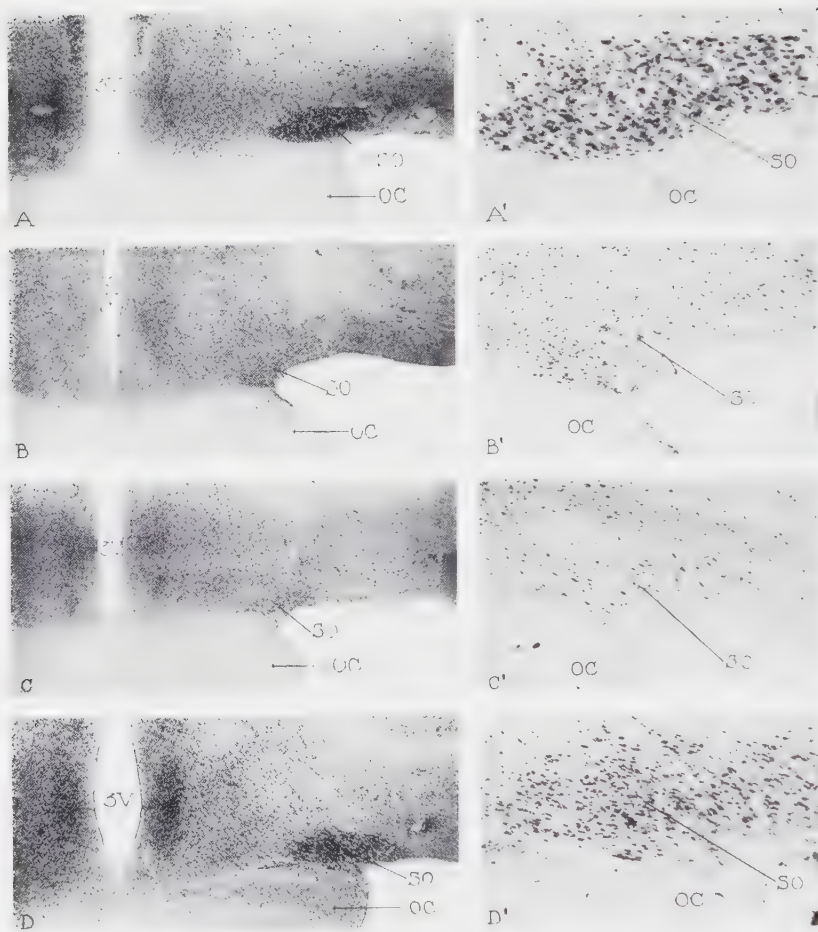


Fig. 2 Photomicrographs of transverse sections through the densest portion of the supraoptic nucleus of: A, A', normal monkey; B, B', monkey with transection of the infundibular stem shown in figure 1A; C, C', monkey with transection of the median eminence shown in figure 1B; D, D', monkey with section of the posterior half of the median eminence shown in figure 1D. Cresyl violet, 10μ , A-D $\times 7$, A'-D' $\times 19$. Abbreviations are as follows: OC, optic chiasma; SO, supraoptic nucleus; 3 V, third ventricle.

After transection of the infundibular stalk below the median eminence (fig. 1A), cell counts in six monkeys revealed that only 8800 to 11,600 cells remained in the supraoptic nucleus, the average figure for the six animals being 10,000 cells (table 1, column 2). Because of the marked cell loss the margins of the nucleus were much less clear-cut than in the normal animal, but in every questionable instance a liberal rather than a conservative boundary was set. It should be pointed out

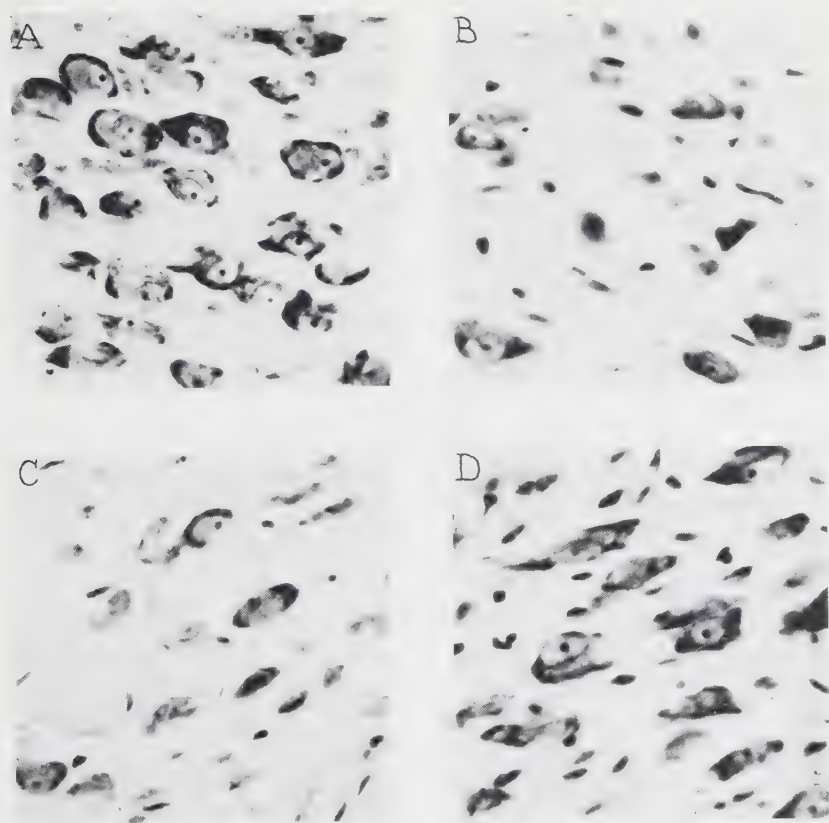


Fig. 3 Photomicrographs of transverse sections through the densest portion of the supraoptic nucleus of: A, a normal monkey; B, monkey with transection of the infundibular stem; C, monkey with transection of the median eminence; D, monkey with section of the posterior half of the median eminence. Cresyl violet, 10 μ . $\times$ 395.

also that these counts included every cell in which a nucleolus could be recognized, though many of the cells counted appeared markedly pathological. Separate estimates of the cells present which were comparable in appearance to those of the normal animal amounted to an average of only 6300 (table 1, column 3). Measurements of the length of a large number of the typical appearing cells which remained in the operated cases indicated, however, that they averaged only about 80% of the length of supraoptic cells of normal, unoperated monkeys. Fewer pathological appearing cells were present in the extreme caudolateral portion of the supraoptic nucleus, but the cell loss was uniformly distributed. Figure 2B shows low and medium power views of a section through the densest portion of the supraoptic nucleus and figure 3B shows the appearance of the supraoptic cells in the animal of this group with section of the stem whose lesion is shown in figure 1A.

After more proximal transections through the median eminence (fig. 1B), the loss of cells was even more marked. In three animals only 5400, 6200 and 6400 cells remained in the supraoptic nucleus (table 1, column 4), and only 1900, 3200 and 4500 of these cells respectively (table 1, column 5) were comparable in appearance to those of the normal animal. Figure 2C shows low and medium power views of a section through the densest portion of the supraoptic nucleus and figure 3C shows the appearance of the cells in the animal with transection of the median eminence whose lesion is shown in figure 1B.

Almost the same amount of degeneration results from section of the anterior portion of the median eminence as after complete interruption, for in the case in which the anterior half alone of median eminence was sectioned (fig. 1C) only 6900 cells remained in the supraoptic nucleus (table 1, column 6). This might be expected for the supraoptico-hypophyseal tract is generally regarded as being located for the most part in the anterior portion of the infundibulum, while the tubero-hypophyseal connections occupy the more posterior portion.

These figures show that after transection of the stalk below the median eminence about 70% of the normal number of cells in the supraoptic nucleus degenerate and disappear, and only about 18% remain which resemble those found in the normal animal. After more proximal transections through the median eminence about 82% of the normal number of cells are missing and only 9% remain which are comparable in appearance to those present normally. After sections through the median eminence a part of the neurohypophysis still remained intact in the median eminence proximal to the section and was probably innervated by some, at least, of the remaining cells. The possibility exists that the axones of other cells remaining in the supraoptic nucleus may have passed elsewhere than into the hypophysis. The results of these counts indicate, however, that fully 85% and probably at least 90% of the normal number of supraoptic cells give rise to hypophyseal connections.

A control is presented by the count in the last column of table 1, from the animal in which an incomplete section was confined largely to the posterior or dorsal half of the median eminence (fig. 1D). The operative procedure was more severe than usual in this case and the lesion was located in immediate proximity to the supraoptico-hypophyseal tract. The tract was largely spared, however, and 27,000 cells remained in the supraoptic nucleus. Figure 2D shows low and medium power views of a section through the densest portion of the supraoptic nucleus and figure 3D shows the appearance of the supraoptic cells in this case. If the degeneration of the supraoptic nuclei in the experiments presented above could conceivably have occurred in some non-specific manner, it should have been present in this instance also, and this case presents complementary evidence that the cell loss in the supraoptic nucleus following complete sections of the median eminence or infundibular stem is a specific retrograde degeneration resulting from interruption of the supraoptico-hypophyseal tract.

The degree of degeneration of the supraoptic nucleus of the operated cases in table 1 is that present 9 to 12 weeks after operation. A study of one animal with stalk section killed 3 weeks postoperatively revealed that a large amount of degeneration had already taken place at this early stage. Except for rare instances of neuronophagia, the degeneration of cells in the supraoptic nucleus has occurred without appreciable increase of neuroglial or connective tissue elements, which is also evidence that it does not take place as a non-specific traumatic effect or through interference with the vascular supply, both of which are known to induce glial and connective tissue proliferation. Walker ('38) has reported that the retrograde degeneration of some thalamic nuclei following lesion of the cerebral cortex also occurs without gliosis.

The degeneration of the supraoptic nuclei is the most striking alteration in the hypothalamus of the monkey following interruption of infundibular connections. Some, but by no means a comparable loss of cells takes place also in the paraventricular nucleus. Though no attempt was made to obtain complete cell counts of the paraventricular nucleus, counts of representative fields revealed a definite reduction of cells following sections of the stem or median eminence as compared to normal. This cell loss may possibly be as great as 20% of the normal, but after such chronic sections a very large part of the paraventricular nucleus remains unaltered. The suprachiasmatic nucleus which has been suggested to have hypophyseal connections was not affected in any of these cases.

It was hoped that the method of retrograde degeneration might give some information regarding the origin of the tubero-hypophyseal tract which passes downward in the posterior portion of the infundibulum, particularly in the case in which it was interrupted almost in isolation. No definite information could be obtained, however, and it may be concluded that in the monkey this tract does not take origin from neurons closely grouped in a well-defined nucleus, as does the supraoptico-hypophyseal tract.

DISCUSSION

In these experiments various alternative explanations which might be suggested for the degeneration of the supraoptic nuclei, such as interruption of blood supply, injury to the hypothalamus, generalized pathological processes, etc., could be definitely excluded, and the loss of cells in the supraoptic nuclei undoubtedly occurred as a retrograde degeneration following interruption of their axones in the infundibular stalk.

It has been pointed out that the number of cells lost was somewhat greater following the more proximal sections through the median eminence than after the more distal sections through the stalk below the median eminence. It is frequently stated that the severity of the retrograde reaction increases the closer to the cell that the axone is interrupted. This generality is apparently based exclusively on studies of retrograde degeneration after peripheral nerve injury, however, and it is questionable whether it can be applied to central nervous connections, to which the supraoptico-hypophyseal tract is analogous. Levin and Bradford ('38) have observed a striking loss of cells in area 4 of the cerebral cortex after contralateral hemisection of the cervical spinal cord, and the results of Walker ('38) and others who have studied the retrograde degeneration of cell groups in the thalamus after extirpation of regions of the cerebral cortex indicate that maximal degeneration may occur following interruption of only the most distal portion of the central axones. It would seem, therefore, that the less extensive degeneration of the supraoptic cells after distal sections of the stalk was due to the preservation of the median eminence which a part, at least, of the remaining cells innervated.

In a recent study in man, Rasmussen ('38) finds that the supraoptic nuclei appear to contain twice as many cells as are necessary to give origin to the fibers in the infundibular stalk. It is likely that the axones of some of these excess cells terminated in the median eminence proximal to the level at which the fiber counts were made. Some excess of supraoptic cells to infundibular fibers may be present in the monkey.

Our counts roughly indicate that about 48,000 fibers from the main body of the supraoptic nucleus might be present in the stalk below the median eminence (34,000 minus 10,000 times 2). There is not a very great discrepancy between this estimate and Rasmussen's ('38) count of 40,000 nerve fibers in the stalk of the monkey, but connections from other cells in the hypothalamus are not taken into account. It should be borne in mind that it is very difficult to differentiate and count unmyelinated fibers. It often happens that two or more fibers lie so close together that they cannot be differentiated and they then would be counted as one. Rasmussen's fiber count may therefore be somewhat too low, and he states that it is probably an under- rather than an over-estimate. It is interesting to note that a comparison of the cell counts of the supraoptic nuclei in the monkey made in the present experiments with those of Rasmussen ('38) in man reveals that the supraoptic nuclei of man contains many more cells than in the monkey, possibly twice as many.

No attempt was made in this study to elucidate the cytopathological alterations which the cells of the supraoptic nucleus undergo as they degenerate and the present experiments have no specific bearing on the work of Gagel and Mahoney ('36) whose results in the monkey were confined to the observation that the cells of the supraoptic nuclei did not show a 'typical retrograde reaction' at any time between 24 hours and 2½ months after hypophysectomy or putting a clip on the stalk. This might be anticipated because of the atypical appearance of the cells of the normal supraoptic nucleus.

These authors do not refer to the marked reduction in the number of cells in the supraoptic nucleus which occurs after chronic interruption of its efferent tract to the hypophysis, but strong evidence that such a reduction had occurred in their three chronic cases is furnished by a photograph of the supraoptic nucleus of one monkey surviving hypophysectomy 2 months (Gagel and Mahoney, '36, Abb. 4, S. 599). A comparison with the photograph of the nucleus of a normal

monkey of the present series (fig. 3A) indicates that the cell density in Gagel and Mahoney's case is greatly reduced as compared to normal, particularly when it is kept in mind that the photograph presented by Gagel and Mahoney is of a section twice as thick (20μ) as that of the present series (10μ).

The skepticism which a few have recently expressed regarding the existence of any prominent connections between the supraoptic nuclei and the hypophysis has been shown to be unwarranted by the results of Ingram and Fisher ('36) in the cat, Rasmussen ('37) in the rat, and Hare ('37) in the dog, all of whom have found the same retrograde degeneration of the supraoptic nuclei after hypophysectomy or section of the infundibular connections which has been described here in the monkey. The recent study of Rasmussen ('38) of hypophyseal innervation in man has shown that the supraoptico-hypophyseal system is certainly not smaller and is probably much larger in man than in lower animals. The quantitative data which have been presented by Rasmussen ('38) for man and monkey and which the present study has added for the monkey should put an end to suggestions that the supraoptico-hypophyseal system has become much reduced in size in the primates. These recent studies confirm an extensive earlier literature which has been reviewed by Fisher, Ingram and Ranson ('38). The comparative neurological aspects of this system have recently been presented from Kapper's laboratory by Boon ('38). It may be said without exaggeration that few connections in the nervous system rest upon the volume of supporting evidence which has been brought forward for that between the supraoptic nuclei and the neurohypophysis.

SUMMARY

Quantitative studies of the supraoptic nuclei of the hypothalamus have been made in the normal monkey and in monkeys in which the infundibular stalk had been interrupted for 9 to 12 weeks.

In the normal monkey the main body of each supraoptic nucleus contains between 30,000 and 40,000 cells.

After transections of the infundibular stalk below the median eminence about 70% of the normal number of supraoptic cells disappear and less than 20% remain which resemble those found in the normal animal.

After transections through the median eminence about 80% of the cells of the supraoptic nucleus are lost and less than 10% remain which resemble in appearance those found normally.

Evidence is presented that this loss of cells occurs as a specific retrograde degeneration following interruption of the supraoptico-hypophyseal tract.

It is concluded that suggestions that the supraoptico-hypophyseal tract is either attenuated, vestigial or altogether absent in the monkey, are not well founded.

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MULTINUCLEATE SYMPATHETIC GANGLION CELLS IN MAN

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ONE FIGURE

During inspection of routine histological material we observed sympathetic ganglia containing many multinucleate cells in a section of normal adult human seminal vesicle. Of 254 cells counted in six small ganglia on this slide, forty-six had two or more nuclei. Cells were found with 2, 3, 4, 5, 6 and 9 nuclei (fig. 1). Subsequently, preparations from four normal adult human prostates, three normal adult human urinary bladders and from one additional specimen of normal adult human seminal vesicle were examined. In all these, sympathetic ganglia containing nerve cells with two, three or more nuclei were found, though not in the abundance noted in the first specimen of seminal vesicle. The sections of prostate showed sympathetic ganglion cells possessing 2, 3, 4, 5 and 7 nuclei; those of bladder had cells with 2, 3, 4 and 5 nuclei; and the slides from the additional seminal vesicle contained cells with two and three nuclei. In all the specimens, there were other large ganglion cells, mononuclear but with redundant cytoplasm, in which serial sections might have revealed multinucleosis. Although it seemed that the nuclei of the multinucleate cells were prone to lie more or less in one plane, the sections inspected were thin and it is possible that serial sections would have revealed neurons containing even more than nine nuclei. Ganglia and their cells showed no pathological changes.

Sympathetic ganglion cells containing two or more nuclei have long been known in certain lower animals, especially

rodents,¹ but the occurrence of such cells in man is not well recognized in this country. Only a few American textbooks of histology and neuro-anatomy mention that binucleate sympathetic ganglion cells may be found in man. Neurologists and anatomists of our acquaintance confessed to complete lack of information concerning sympathetic multinucleosis.

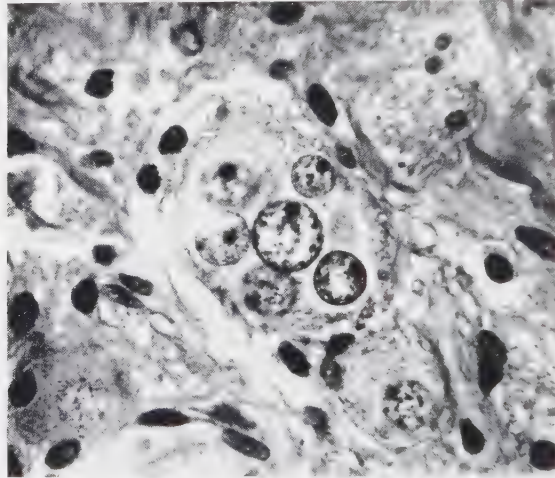


Fig.1 Sympathetic ganglion cells from one of the intrinsic ganglia of the adult human seminal vesicle. Of nine nuclei present in the large cells, six are in focus and a seventh can be made out as a dim shadow to the right of the large central nucleus. Two mononucleated ganglion cells of the usual size may be seen below the large one. Photomicrograph, $\times 900$; Mallory-azan stain.

¹ Multinucleate sympathetic ganglion cells have been observed in the rabbit (Guye, 1866; Schwalbe, 1868; Apolant, 1896; Michailow, '08; Müller, '09; Cajal, '11; Carpenter and Conel, '14; Uenae, '30), guinea pig (Schwalbe, 1868; Apolant, 1896; Carpenter and Conel, '14; Roussy and Mosinger, '36), muskrat and porcupine (Carpenter and Conel, '14). In the rabbit, binucleate cells are the rule; Matsui ('26) found that they constituted 89% of the cells in the stellate ganglion. Such cells are, however, only very rarely found in the rat (Apolant, 1896; Carpenter and Conel, '14; Matsui, '26), mouse (Apolant, 1896; Carpenter and Conel, '14), squirrel (Apolant, 1896), thirteen-lined gopher and prairie dog (Carpenter and Conel, '14).

In other mammals, sympathetic multinucleosis, though said to be rare, has been encountered in the horse (Michailow, '08), dog (Mayer, 1872; Matsui, '26; Waddell '29), cat (Mayer, 1872; Matsui, '26; Uenae, '30), and porpoise (Mayer, 1872). Multinucleate cells have likewise been observed in the frog (Mayer, 1872; Dehler, 1895; Cole, '25).

This may be due to the infrequency of microscopic examination of the pelvic sympathetic ganglia but it is nevertheless astonishing, in view of the recurrent records of this phenomenon in the continental European literature.

More than one nucleus has been found in human sympathetic ganglion cells by no less than seventeen investigators and the list of references we have assembled may not be complete. Bielschowsky ('28) states that Remak as early as 1854 and Ranvier in 1878 noted binucleate sympathetic ganglion cells under normal conditions. Mayer (1872) likewise observed binucleate sympathetic neurons in man. Marinesco (1898) illustrated a trinucleate cell from the superior cervical sympathetic ganglion, and Sobotta ('02) pictured binucleate and trinucleate cells in a ganglion in the neighborhood of the seminal vesicle. In 1903 Georgiewski ('30) found cells in the ganglia of the prostate with two to fourteen nuclei. Müller ('09) stated that the thoraco-lumbar chain may, though rarely, exhibit binucleate ganglion cells. Hryntschak ('23) found cells with two, three or even more nuclei in ganglia of the urinary bladder. Münzer ('26) saw binucleate and trinucleate neurons in the sympathetic plexus of the seminal vesicle. Herzog ('26) observed them with six to eight nuclei in newborn individuals. It is averred by Stohr ('28) that binucleate sympathetic cells are common and cells with more than two nuclei no rarity. Watzka ('28) found them with two to fourteen nuclei in the ganglia near the seminal vesicle and Penitschka ('29) observed many with two or more nuclei in the cervico-uterine ganglia. De Castro ('32) declares that binucleate cells are often seen and that he has found sympathetic neurons with three, four and five nuclei in fetuses; the latter cells he believes to be abnormal. Baron ('32) demonstrated cells with more than one nucleus in the ganglia of the rectum, seminal vesicle and thoraco-lumbar chain. Contrary to observations of Matsui ('26), who could find no binucleate cells in the cervical sympathetic ganglia of man, Baron states that all sympathetic ganglia have considerable numbers of binucleate cells.

The significance of sympathetic multinucleosis is not clear. De Castro ('32) considers cells with more than two nuclei to be abnormal. Roussy and Mosinger ('36) working with guinea pigs and Sunder-Plassmann ('33) experimenting with rabbits thought multinucleate cells evidence of distorted physiology. Sunder-Plassmann's research has been thoroughly discredited by Harting ('35). The apparent constancy of occurrence of these cells would seem to preclude interpreting them as pathological. In animals, Schwalbe (1868), Apolant (1896) and Uenae ('30) have found that multinucleosis increases with age² but de Castro ('32), Herzog ('26), Kölliker (1896) and Spiegel and Adolph ('22) have observed multinucleate cells more commonly in the young than in the adult. Spiegel and Adolph believe that they are reserve cells which can undergo division without further nuclear changes and most authors hypothesize that binucleate cells result from incomplete, probably amitotic,³ cell division. On the other hand Cole ('25) believes that the binucleate sympathetic cells of the frog are due to fusion of adjacent cells and Kuntz ('34) thinks that the coupled sympathetic neurons seen by Waddell ('29) in the dog may be included in the same category.

Whatever may be the significance of multinucleosis, it is clear to us that it must be considered a phenomenon of rather frequent occurrence in adult man, at least in the sympathetic ganglia adjoining the bladder, prostate and seminal vesicle. The observation of such elements should not be taken as evidence of pathology.

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² Andrews ('39) finds that the cerebellar Purkinje cells of senile mice tend to exhibit the binucleate condition.

³ Roussy and Mosinger ('35) believe that there is evidence of amitotic regeneration in the binucleate cells of the human hypothalamus.

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THE NATURE OF THE X-ZONE OF THE ADRENAL GLAND OF THE MOUSE

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FOUR PLATES (TWENTY-ONE FIGURES)

The adrenal gland of the mouse is characterized by the existence during early life of a zone of tissue variously designated as the X, fetal, interlocking or androgenic zone. This zone differs in many respects from the remainder of the cortex. At the height of its development in both sexes the X-zone consists of a compact segregated group of cells which lies between the zona fasciculata and the medulla and occupies from 12 to 55% of the total volume of the cortex in different strains of mice (Howard, '38). In dwarf mice (Deanesly, '38) the X-zone is entirely lacking. The cells of this zone are smaller than the other cortical cells and their cytoplasm stains more deeply (Howard, '27; Whitehead, '33). Like other portions of the cortex the X-zone contains an appreciable amount of ascorbic acid (Leblond and Gardner, '38); unlike them, it has little or no histologically demonstrable lipid (Howard, '27; Whitehead, '33; Cramer, '28). The zone is first recognizable during the second week of extra-uterine life. Thereafter it increases in thickness until 3 to 4 weeks of age. The subsequent development of the zone differs in the two sexes. In the male, further growth ceases and degeneration begins, so that at 4 to 5 weeks of age the X-zone is definitely smaller than in the female; it disappears entirely by the end of the seventh to eighth week of life. Its site is marked thereafter by a narrow band of perimedullary

connective tissue (Howard, '27; Deanesly, '28; Waring, '35; Starkey and Schmidt, '38). In the female the X-zone continues to grow, and reaches the peak of its development at 5 to 6 weeks of age. It persists in the unmated mouse throughout almost its entire reproductive period, disappearing slowly toward the end of this time. In the mated female the zone degenerates rapidly during the first 12 days of gestation to be replaced by a connective tissue septum (Masui and Tamura, '24; Howard, '27; Deanesly, '27; Waring, '35; Starkey and Schmidt, '38).

Castration of the male during the existence of the X-zone causes its persistence and continued growth until it reaches the proportions found in the unmated female. On the other hand, ovariectomy does not influence the course of development of the zone (Masui and Tamura, '24; Howard, '30; Deanesly, '28; Martin, '30; Starkey and Schmidt, '38). The administration of testosterone or other androgenic steroids causes a rapid disappearance of the X-zone in normal and in castrated male as well as in immature, nulliparous and ovariectomized female mice. When androgenic substances are injected before the normal appearance of the zone, its development is inhibited (Martin, '30; Cramer and Horning, '37; Deanesly and Parkes, '37; Starkey and Schmidt, '38). The effects of other extracts (hypophyseal, ovarian, thyroid) have also been studied but the results described are conflicting and difficult to interpret (Preston, '28; Burrows, '36; Martin, '30; Cramer and Horning, '37; Lacassagne and Raynaud, '37, p. 1183).

From the above-described facts, two chief interpretations of the significance of the X-zone have arisen. One group of investigators believes that the cells of the X-zone constitute true cortical tissue which subserves the same function as does the rest of the adrenal cortex (Masui and Tamura, '24; Deanesly, '28; Preston, '28; Whitehead, '33; Waring, '35; Leblond and Gardner, '38). A second group have suggested that the X-zone may exert some sexual function in normal

mice¹ (Grollman, '36; Howard, '38). The striking degeneration and the inhibition of the development of the X-zone induced by testosterone support the latter view.

Neither of the above-mentioned theories regarding the nature and significance of the X-zone is based on conclusive data. The fact that the cells of the X-zone are rich in vitamin C is no indication of their cortical activity since this vitamin is found also in high concentration in the adrenal medulla as well as in other tissues (hypophysis, thymus, corpus luteum, etc.). The observation that there is no X-zone in dwarf mice is also not pertinent. The extent of development of the X-zone has been found to vary so widely in different strains, that the absence of X-zone in these animals may be regarded as merely another strain difference. A more pertinent argument (Preston, '28) is the fact that the administration of thyroxin for long periods of time results in the hypertrophy of the X-zone of both sexes, in a marked increase in the amount of lipid in the cells of this zone, and in its persistence after the time when it would have regressed in untreated animals. It has since been shown in other animals whose adrenals are devoid of an X-zone, including the rat (Kliwanskaja-Kroll, '28), rabbit, cat and guinea pig (Habán, '38) that the prolonged administration of thyroid preparations leads to an hypertrophy of the cells of the zona fasciculata and zona reticularis and to an increase in their content of visible lipid. It would seem that the elements of the X-zone of the mouse respond in the same way to metabolic stimulation as do the reticularis cells of species devoid of this morphological zone.

The view that the X-zone exerts an androgenic function offers an interesting postulate which would satisfactorily explain many well-established observations (Grollman, '36). However these observations offer no conclusive proof of this theory. Thus, as suggested by Leblond and Gardner ('38),

¹ A third interpretation offered by Cramer and by Cramer and Horning is that the X-zone is chromaphil rather than cortical tissue. There is nothing in the development of this zone, or in its response to stimuli that would support such a view.

the inhibitory effect of testosterone on the X-zone may be mediated through the depressing effect of this androgen on the hypophysis. Similarly, the degeneration of the X-zone that occurs normally in the male at the time when the testis begins to function may not be caused by 'an atrophy of dis-use,' but may be secondary to the general metabolic changes occurring in the organism at puberty. An androgenic activity of the X-zone has been claimed also (Grollman, '37; Howard, '38) on the basis of the observations that certain accessory male sex organs will continue to grow for a short time after castration if the testes are removed within the first 2 weeks of life. This residual growth has been attributed to an androgenic activity of the X-zone. However, this assumption fails to explain why the sex organs in rats and mice castrated during the first 2 weeks of life do not exceed the usual castrate level in spite of the persistence and hypertrophy of the X-zone in the adrenal glands of such animals. Moreover, it has been shown that the same delay in the degeneration of some of the accessory sex organs takes place in the entire absence of X-zone tissue (Gersh and Grollman, '39). Further evidence in support of the assumption of an androgenic function of the adrenal is based on the fact that the time when the X-zone develops rapidly in the mouse coincides with the period when the accessory sex glands show a disproportionate rate of growth (Howard, '38). This argument, however, loses its validity in the face of the demonstration that the same rate of growth of at least some of these glands is maintained in the complete absence of X-zone tissue (Gersh and Grollman, '39).

The present paper describes the gross and cytological changes which the X-zone undergoes in response to changes in the hormone output of the adrenal induced by the administration of thyroid extract or adrenal cortical hormone. The administration of thyroid extract causes an hypertrophy of the cells of the X-zone and of the adrenal gland as a whole. The administration of cortical hormone, on the other hand, results in the atrophy or almost complete disappearance of

the X-zone. The conclusion to which we are led is that the X-zone produces the same cortical hormone that is produced by the other portions of the adrenal gland. It differs from other zones of the cortex in that it reacts less readily to stimulation, and is depressed more easily. The attitude adhered to in this paper is that there is but one hormone secreted normally by the adrenal cortex (Grollman, '36). The amount of this hormone secreted is delicately adjusted to the activity of the organism.

MATERIAL AND METHODS

Three series of experiments were performed. In the first, normal litter-mate mice of either sex were separated at 3 weeks of age into three groups: a) control mice; b) mice in whose diet was incorporated desiccated hog thyroid powder (containing 0.545% of organic iodine); and c) mice in whose diet was incorporated 1 to 2 units per mouse per day of a charcoal adsorbate of the adrenal cortical hormone. The thyroid powder constituted $\frac{1}{2}$ to 1% of the food consumed by the animals. At least two mice in each group were killed by decapitation at intervals of $3\frac{1}{2}$ days and 10 days after the beginning of the experiment. In the second series of experiments, male mice were castrated at the age of 3 weeks to permit the X-zone to develop to the dimensions observed in young female mice. At 5 weeks of age, the mice were separated into three groups and treated with thyroid or adrenal extract as in the case of the immature mice described above. At least two mice in each group were killed by decapitation 1 or 2 weeks after the beginning of the administration of the hormones. In the third series of experiments immature mice were treated with relatively large doses of adrenal cortical extract or desoxycorticosterone acetate and their adrenals compared with untreated litter-mate controls. All together a total of eighty mice were used.

The adrenal gland of the left side was fixed in Maximow's fluid. Before immersion in the fixative, one end of the gland was cut off to permit more ready penetration into the inner

parts of the organ. It was found subsequently that the reduction of osmic acid in cells adjacent to the cut surface was never greater than in cells farther removed from this area. The right gland was fixed in formalin-Zenker (without acetic acid). Subsequent examination of sections of material from both glands confirmed the findings of other workers that the X-zone in both adrenal glands of mice are in the same stage of activity. The gland fixed in Maximow's fluid was post-osmicated as described elsewhere. Together with the other organ fixed in formalin-Zenker, it was embedded in 60 to 62°C. paraffin and sectioned serially at 5 μ . The sections of the osmicated material were mounted on slides and examined unstained. Alternate slides of sections of the other material were stained in haematoxylin and eosin and in Mallory's triple stain. For strict comparative work, as in the photomicrographs of plates 1 to 4, sections through the widest part of the gland were used.

RESULTS

Effects of thyroid stimulation on the X-zone. In immature mice, the administration of thyroid powder for 3½ days results in a very marked hypertrophy of the X-zone as demonstrated in figures 1 and 2. The cells increase enormously in volume, apparently because of the accumulation in the cytoplasm of large numbers of visible fat droplets which reduce osmic acid. It can be seen in figure 14 that these droplets are spherical and of small, more or less uniform, dimensions, and that they are packed very closely in the cytoplasm. They are so densely crowded that they may appear in many cells in the photomicrograph as one large vacuole, even in a section as thin as 5 μ , due to the effects of overlay. In the control preparations, at this age (fig. 13), there is no visible lipoid. With very high magnifications, small black granules may appear in the cytoplasm. They are highly irregular in shape and size. They may be present in large numbers in some cells while absent in adjacent cells. In preparations fixed in formalin-Zenker, there are no 'holes' or 'shadows' in

the cytoplasm of these cells. Hence the irregular black granules are considered as being due to the reduction of osmic acid by cytoplasmic reducing substances other than those found in visible lipid droplets. In subsequent descriptions, these variable granules of reduced osmic acid will be referred to as 'black granules,' in contradistinction to the visible lipid droplets that are recognizable as morphological elements. In the experimental animals, many cells of the X-zone which are devoid of lipid droplets contain black granules. It should be noted that the X-zone cells are scattered throughout the medulla and can be recognized as cortical cells in stained sections of both the normal and experimental animals.

The above-described hypertrophy of the X-zone is maintained after the administration of thyroid powder for 10 days (figs. 4 and 5). It should be noted that the cells of the X-zone in figure 5 are rich in visible osmic acid-reducing lipoids in spite of their almost complete absence in the remaining portions of the cortex. There are no black granules in the cytoplasm of the cells of any portion of the cortex.

In castrate mice, a similar hypertrophy of the X-zone is produced by the administration of thyroid extract. Treatment for 1 week results in a marked hypertrophy of the cells of this zone. As compared with the control preparation (figs. 7 and 19), large numbers of lipid droplets appear in the cytoplasm of the hypertrophied cells. There is also a marked increase in the number of black granules in the cytoplasm (figs. 8 and 20). It will be seen also in figure 20 that many of the hypertrophied cells contain both lipid droplets and black granules. After treatment for 2 weeks, the hypertrophy of the X-zone is even more apparent. As compared with the control specimen (figs. 10 and 16), the amount of visible fat is enormously increased. The dense black appearance of the cells in figures 11 and 17 is ample evidence that the visible lipoidal granules in the cytoplasm are very closely packed. There is no visible lipid in the control preparation (fig. 16); the amount of visible fat is enormously increased. The dense black appearance of the cells in figures 11 and 17 is ample

evidence that the lipoidal granules in the cytoplasm are very closely packed. There is no visible lipoid in the control preparation (fig. 16), the osmium apparent in the photomicrograph being confined entirely to the irregularly disposed black granules.

Effects of the administration of cortical hormone on the X-zone. In immature mice treated for $3\frac{1}{2}$ days, the X-zone persists (fig. 3) but shows definite signs of the slow atrophy that has been described as taking place normally in the course of the disappearance of this zone. The cells (fig. 15) are smaller and flatter than in the control preparations, especially those adjacent to the medulla. The remaining cells of the X-zone gradually assume the shape of those in the cortex as the latter are approached. There is no visible lipoid in the cytoplasm, and only a few cells contain scattered black granules in their cytoplasm. The continued treatment of immature mice with cortical hormone for 10 days results in the almost complete disappearance of the X-zone (fig. 6). The site of the X-zone is occupied by connective tissue fibers in whose meshes are enclosed a few scattered, elongated cells. Many of these are probably macrophages.

A similar atrophic degeneration of the X-zone takes place in castrate mice 1 week after the continuous oral administration of cortical hormone. The X-zone is reduced to a perimedullary septum of connective tissue fibrils which encloses a thin discontinuous layer of elongated cells (fig. 21). Some of these cells appear to be macrophages. Continued oral administration of cortical hormone for 2 weeks results in similar atrophic changes in the X-zone. In one case, however (figs. 12 and 18), the cells of the X-zone appear to be in an intermediate stage of degeneration, being reduced in size. Those adjacent to the medulla are somewhat elongated. As they approach the zona fasciculata, they tend to become somewhat larger, though they never quite attain the dimensions of the cells in that layer. The cells of the X-zone of all treated castrate mice contain only rarely any visible lipoid (see fig.

21). The number of black granules in the cytoplasm of these cells is smaller than in preparations from control castrates.

The inhibition of development of the X-zone by administration of cortical hormone. A group of fifteen mice were treated with 1) an adrenal cortical extract administered orally, 2) an adrenal cortical concentrate injected intraperitoneally, and 3) a solution of desoxycorticosterone acetate² in sesame oil injected intraperitoneally. The latter is a hydroxy derivative of progesterone which manifests adrenal cortical activity. The adrenals of these animals were compared with those of thirteen untreated litter-mate controls matched for age and sex. As seen in table 1, these procedures result in the inhibition of the development of the X-zone if treatment is begun before this zone begins to develop, or in its premature disappearance when this zone is present at the time therapy is instituted.

The reducing lipid droplets in the glomerular and fascicular zones. An examination of sections of the normal and of the control castrate glands (e.g., figs. 1, 4, 7, and 10) shows that there may be a great variability in the amount of osmic acid reduced by the lipid droplets in the cells of these zones. Usually some osmic acid is reduced, but in at least four specimens, reduction occurred. When the amount of osmic acid reduced by lipid droplets is small, it is to be found in the zona fasciculata adjacent to the X-zone. As the amount of such lipid increases, an increasingly larger part of the fasciculata contains it. It spreads finally into the cells of the zona glomerulosa, where in one case it was found in the cells adjacent to the capsule.

After the administration of thyroid powder, the number of visible reducing lipid droplets tends to increase (figs. 2, 5, 8 and 11). But there are specimens in which no reducing lipoids are visible under these conditions. After the administration of cortical hormone in moderate overdosage the number of visible droplets which reduce osmic acid may be

² We are indebted to Dr. Ernst Oppenheimer of the Ciba Company for kindly supplying us with the desoxycorticosterone acetate.

greatly increased in number (figs. 3 and 12). However, there may be instances when very little lipoid of this sort is present. Under these conditions, the droplets may be present in cells in the deeper parts of the fascicular zone or in cells of the zona glomerulosa.

Total lipoidal droplets in the glomerular and fascicular zones. Fixation of the adrenal glands preserves adequately the space occupied by the lipoidal droplets. It is, therefore,

TABLE 1

The inhibition of the development of the X-zone by the administration of adrenal cortical hormone and desoxycorticosterone acetate

LITTER NUMBER	ANIMAL NUMBER	AGE, DAYS	TREATMENT	STATE OF X-ZONE
1	3, 4 ¹	26	CHA for 7 days	Absent
1	5, 6 ¹	35	CHA for 16 days	Absent
1	7, 8 ¹	42	CHA for 16 days, no treatment 7 days	Absent
2	13, 14 ²	19	DOCS for 7 days	Absent in three glands; narrow in one gland
3	19 ²	11	DOCS for 8 days	Absent in one gland, present in spots in the other
3	20 ²	16	DOCS for 13 days	Extremely narrow zone
3	21 ²	21	DOCS, for 11 days; no treatment for 7 days	Absent (whole cortex depressed)
4	27 ³	9	CHC for 6 days	Absent
4	28 ³	14	CHC for 11 days	Presence doubtful
4	29 ³	21	CHC for 11 days; no treatment for 7 days	Absent (whole cortex depressed)

CHA designates that the mice ingested 2 to 3 rat units of cortical hormone as a charcoal adsorbate (Grollman). DOCS designates that the mice were injected daily intraperitoneally with 0.5 mg. desoxycorticosterone acetate dissolved in sesame oil. CHC designates that the mice were injected intraperitoneally daily with 0.1 mg. of an active concentrate of adrenal cortical hormone containing 2 rat units.

¹ Litter-mate untreated controls matched for sex, when the treatment was begun, had a large X-zone which was present at the beginning of treatment (19 days).

² Litter-mate untreated controls matched for sex all showed an appreciable X zone.

³ Litter-mate untreated controls matched for sex showed traces of an X-zone at 5 and 9 days of age and a large X-zone at 14 days of age.

possible to compare the relative amounts of such material present in the cells. As demonstrated by Hoerr the lipid droplets in any given cell have similar dimensions. In general, these droplets are larger in the cells of the glomerular and fascicular zones than in those of the X-zone. Stimulation of the gland by thyroid administration results in a uniform decrease in the size of the droplets in numerous cells of the two former zones. With few exceptions, the cells of the glomerular zone contain many more lipid droplets in the thyroid-treated animals than are visible in the control preparations.

A very striking result of thyroid stimulation of the adrenal glands are the wide, lipid-free zones which appear in different parts of the zona fasciculata. In some animals this 'clear' zone includes the peripheral fascicular region, encroaching somewhat on the glomerulosa; in others it is confined to the middle portion or to the region adjacent to the X-zone; while in still others it is limited to narrow regions on the periphery of the zona fasciculata and adjacent to the X-zone. Though the cells in these clear zones are free of visible lipid and their cytoplasm appears to be dense, they may be larger than most of the hypertrophic cells of this region whose cytoplasm is crowded with droplets. Such enlarged cells cannot be found in preparations from control animals. Of the animals treated with cortical hormone, only one had a large clear zone in the zona fasciculata.

SUMMARY AND DISCUSSION

The stimulation of the adrenal glands of immature normal or castrate mice by the administration of thyroid extract results in an increase in the size of the cells of the X-zone and in an accumulation in the cytoplasm of large numbers of small, even-sized, spherical droplets of lipid that reduce osmic acid. In the outer layers of the cortex there is usually an increase in the number of cells containing lipid or visible droplets. At the same time, the cells of a portion of the zona fasciculata become hypertrophied, in spite of the complete

absence of visible lipid. The cytoplasm of these cells appears to be dense and not highly vacuolated.

The 'lipoid' hypertrophy of the X-zone after stimulation with thyroid extract has been described by Preston ('28). A similar hypertrophy of the fascicular and reticular zones has been reported in species (rats, cats, rabbits and guinea pigs) which lack an X-zone. The behavior of the X-zone under this form of stimulation, then, simulates that of the true cortex.

Preston noted that some of the hypertrophied cells of the X-zone appear to fuse, with the formation of large lipoidal vacuoles. In our material, such large fused cells were never observed. Even in their most extreme state of hypertrophy, the cells of the X-zone maintained their own individuality, and the lipoidal droplets of their cytoplasm remained discrete. In view of the difficulty in preserving the lipid of the cells of the adrenal glands, the large fused cells observed by Preston must be interpreted as poorly fixed ones whose cytoplasm had been disrupted by the post-mortem coalescence of small droplets of lipid.

Although the injection of oestrogenic substances for a short time has no effect on the X-zone, their prolonged administration leads to the formation of the above-described giant fused cells (Burrows, '36; Lacassagne and Raynaud, '37, p. 1186). The latter observers also found that the administration of polonium salts, which are highly toxic, also leads to the same result. In view of our findings, the giant cells present in the X-zone after the administration of oestrogens may be interpreted as post-mortem artifacts similar to those described by Preston. The hypertrophy may also be regarded as evidence of the toxicity of the oestrogens (Tislowitz, '38) when administered over long periods of time in large doses and of a disturbed metabolism of the animal. The response, in other words, is a non-specific one, and is of the same nature as that induced by the administration of polonium salts. The conflicting results of Martin ('30) are too confusing to enter into the present discussion and require confirmation.

Lacassagne and Raynaud ('37) also point out that the adrenal glands of mice treated for several months with oestrogenic compounds present an atrophic appearance. The cells of the zona fasciculata become enlarged, free of lipoid, and contain a dense, strongly acidophilic cytoplasm. These cells correspond to the clear portions of the zona fasciculata observed in glands stimulated by thyroid extract. This is further evidence that under prolonged treatment, oestrogens act on the adrenal glands of the mouse as do other substances which exert a deleterious action on the organism.

The administration of small amounts of cortical hormone to immature or castrate mice leads to a depression of the X-zone in the adrenal glands of these mice. This takes the form of an atrophic degeneration which may lead to almost complete disappearance of the zone and in very young animals to the prevention of its development. The reaction is similar to the atrophic condition of thyroid gland cells induced by the administration of thyroxin. The same reaction for true cortical cells has been demonstrated in the adrenal gland of the rat, to which massive doses of the hormone had been administered (Ingle, Higgins and Kendall, '38). When cortical hormone is administered to mice before the X-zone has begun to develop, it suppresses its appearance (table 1).

The behavior of the X-zone in response to the administration of thyroid powder and of cortical hormone supports the view that the X-zone subserves the same function that is performed by the glomerular and fascicular zones of the adrenal gland. The X-zone might be considered as less active cortical tissue which reacts to an increased demand of the organism for cortical hormone. Evidence will be presented in a forthcoming volume of the Contributions to Embryology, Carnegie Institution of Washington, that the X-zone is the most poorly vascularized region in the adrenal cortex. We are thus led to the belief that the cells of the X-zone secrete cortical hormone in the normal animal, and act as a source of this hormone particularly when the need for it is greater than usual.

CONCLUSION

When the adrenal gland of the immature or castrate mouse is stimulated by the administration of thyroid powder, which creates a need for cortical hormone in the organism, there is a pronounced hypertrophy of the X-zone. The cells of this zone increase in size and their cytoplasm accumulates large numbers of small droplets of lipoid that reduce osmic acid. Degeneration of the X-zone almost to complete extinction follows the administration of small doses of cortical hormone to immature or castrate mice. In very young animals, administration of large doses of the hormone may inhibit entirely the appearance of the zone. These results are interpreted to indicate 1) that the X-zone performs the same functions that are carried out by the other two zones of the cortex, 2) that the X-zone acts as a reserve tissue which responds to increased demands on the adrenal glands for their hormone, and 3) that the X-zone is that portion of the cortex which is most readily depressed when a great need for cortical hormone does not exist or is satisfied in some other way.

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PLATE 1

EXPLANATION OF FIGURES

Low power photomicrographs through the widest part of the adrenal gland to show the effect on the X-zone of the continuous administration by mouth of thyroid extract and of adrenal cortical hormone adsorbate. All preparations fixed in Maximow's fluid, sectioned at 5μ and photographed unstained. $\times 70$. C, zona glomerulosa and zona fasciculata; X, X-zone; M, medulla.

- 1 Untreated unoperated control mouse. Age 3 weeks.
- 2 Treated unoperated mouse. Thyroid administered for $3\frac{1}{2}$ days. Age 3 weeks.
- 3 Treated unoperated mouse. Cortical hormone administered for $3\frac{1}{2}$ days. Age 3 weeks.
- 4 Untreated unoperated control mouse. Age 4 weeks.
- 5 Treated unoperated mouse. Thyroid administered for 10 days. Age 4 weeks.
- 6 Treated unoperated mouse. Cortical hormone administered for 10 days. Age 4 weeks.
- 7 and 10 Untreated control mice castrated when 3 weeks old. Age 6 weeks (fig. 7) and 7 weeks (fig. 10).
- 8 Treated castrate mouse. Thyroid administered for 7 days. Age 6 weeks.
- 9 Treated castrate mouse. Cortical hormone administered for 7 days. Age 6 weeks.
- 11 Treated castrate mouse. Thyroid administered for 14 days. Age 7 weeks.
- 12 Treated castrate mouse. Cortical hormone administered for 14 days. Age 7 weeks.

Note that the administration of thyroid extract results in a hypertrophy of the cells of the X-zone, which become rich in lipid, whereas the administration of cortical hormone causes a depression of the X-zone.

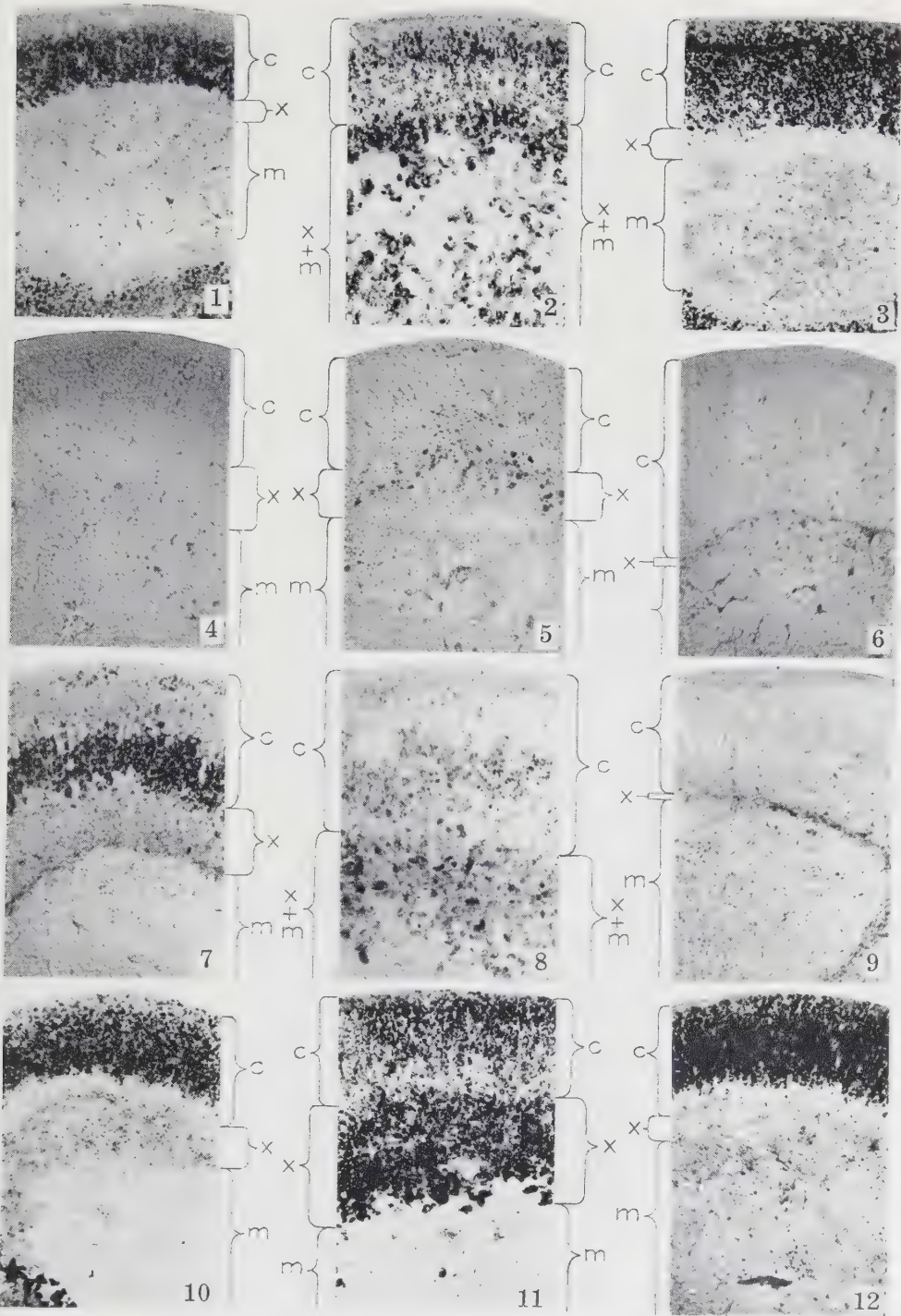


PLATE 2

EXPLANATION OF FIGURES

High power photomicrographs of the same sections as in plate 1 to show in detail the distribution in the cells of the X-zone of visible osmium-reducing lipid and of other reduced osmium particles. $\times 475$.

- 13 Same section as in figure 1.
- 14 Same section as in figure 2.
- 15 Same section as in figure 3.

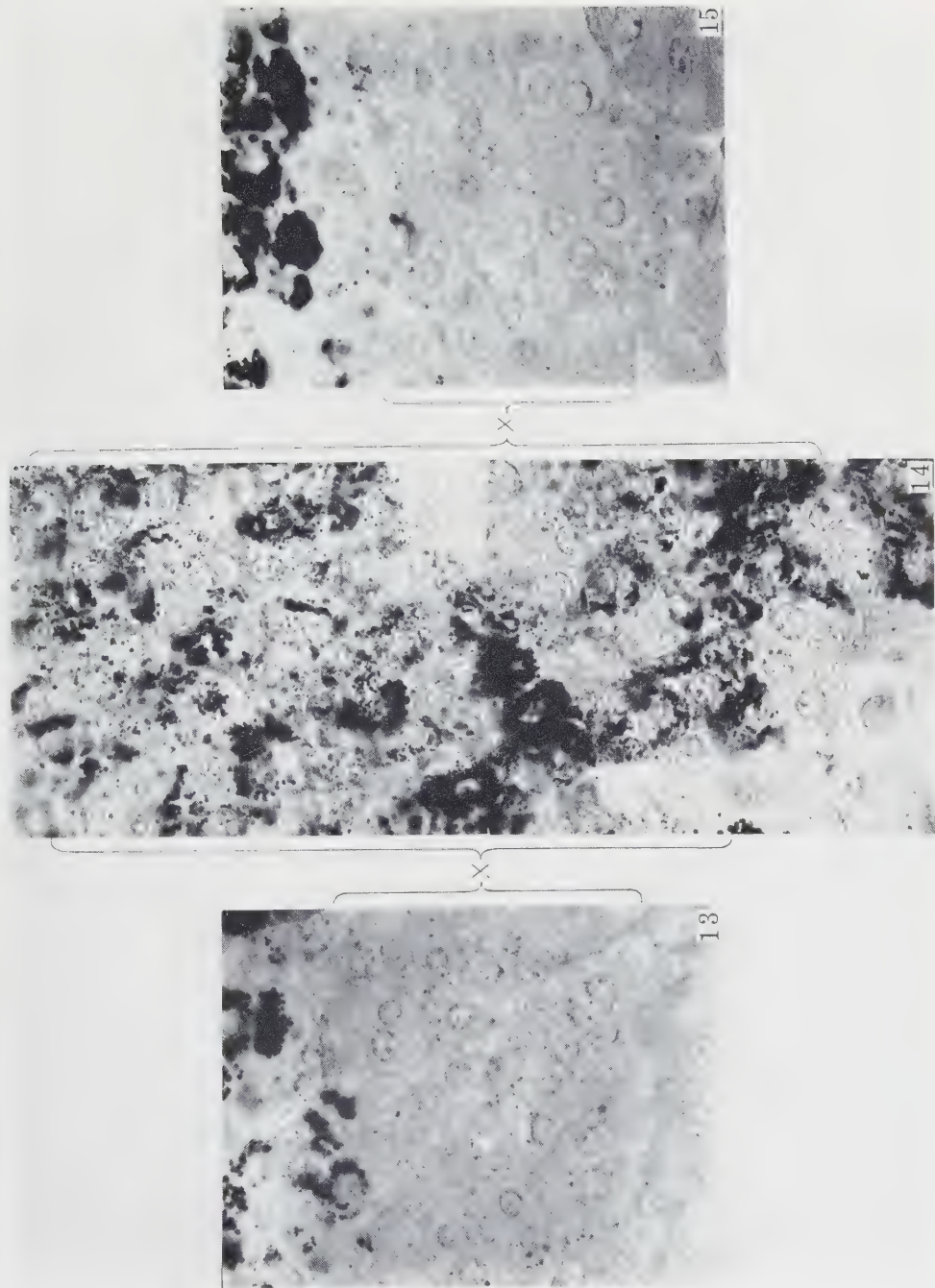


PLATE 3

EXPLANATION OF FIGURES

High power photomicrographs of the same sections as in plate 1 to show in detail the distribution in the cells of the X zone of visible osmium-reducing lipid and of other reduced osmium particles. $\times 475$.

- 16 Same section as in figure 10.
- 17 Same section as in figure 11.
- 18 Same section as in figure 12.

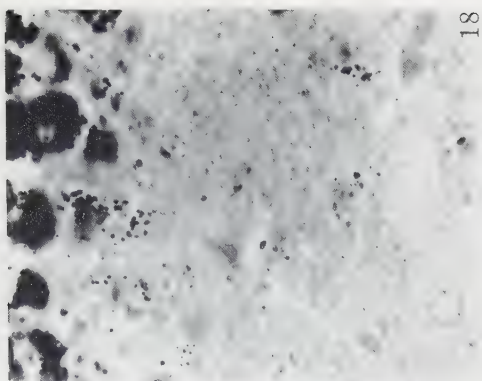
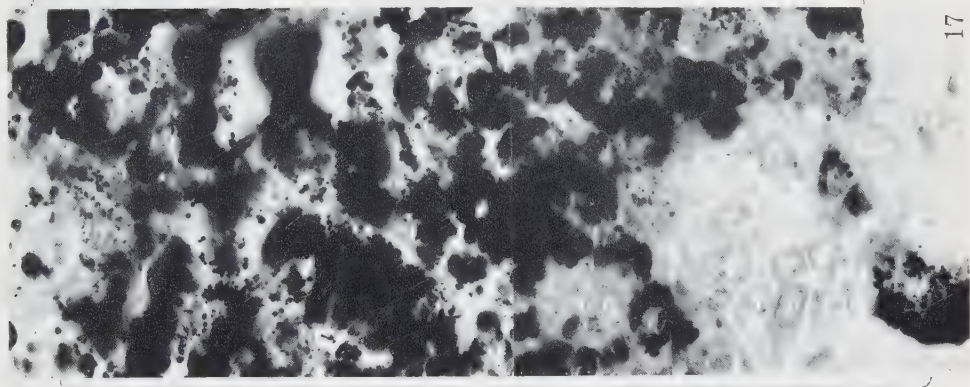
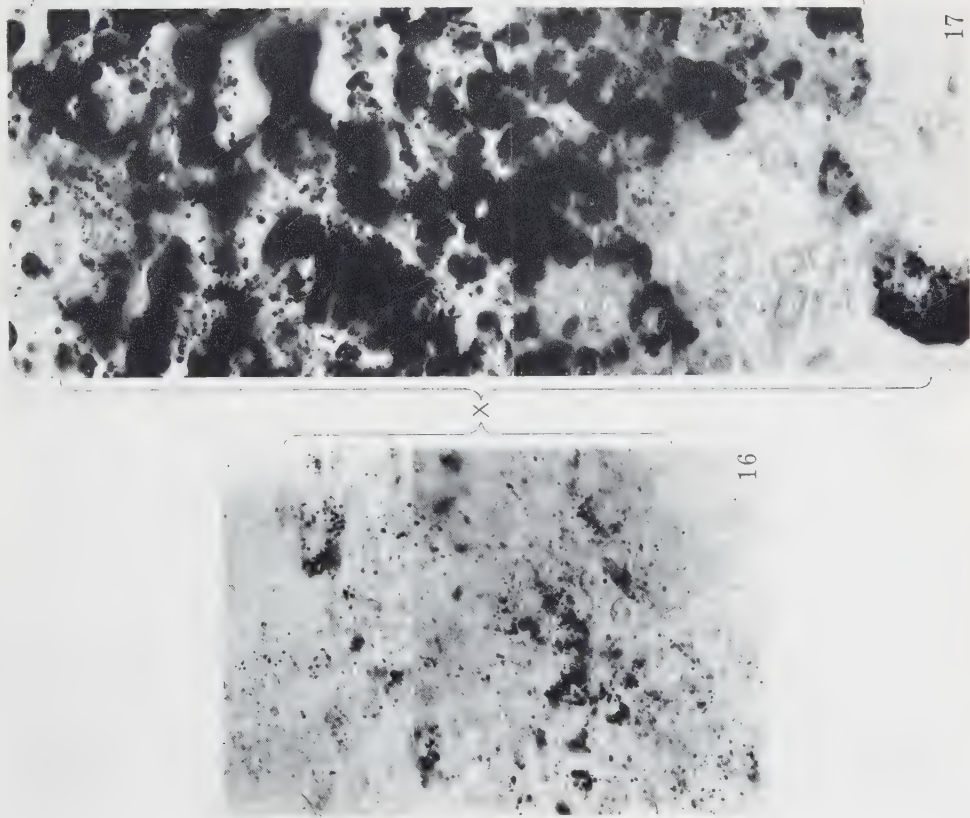
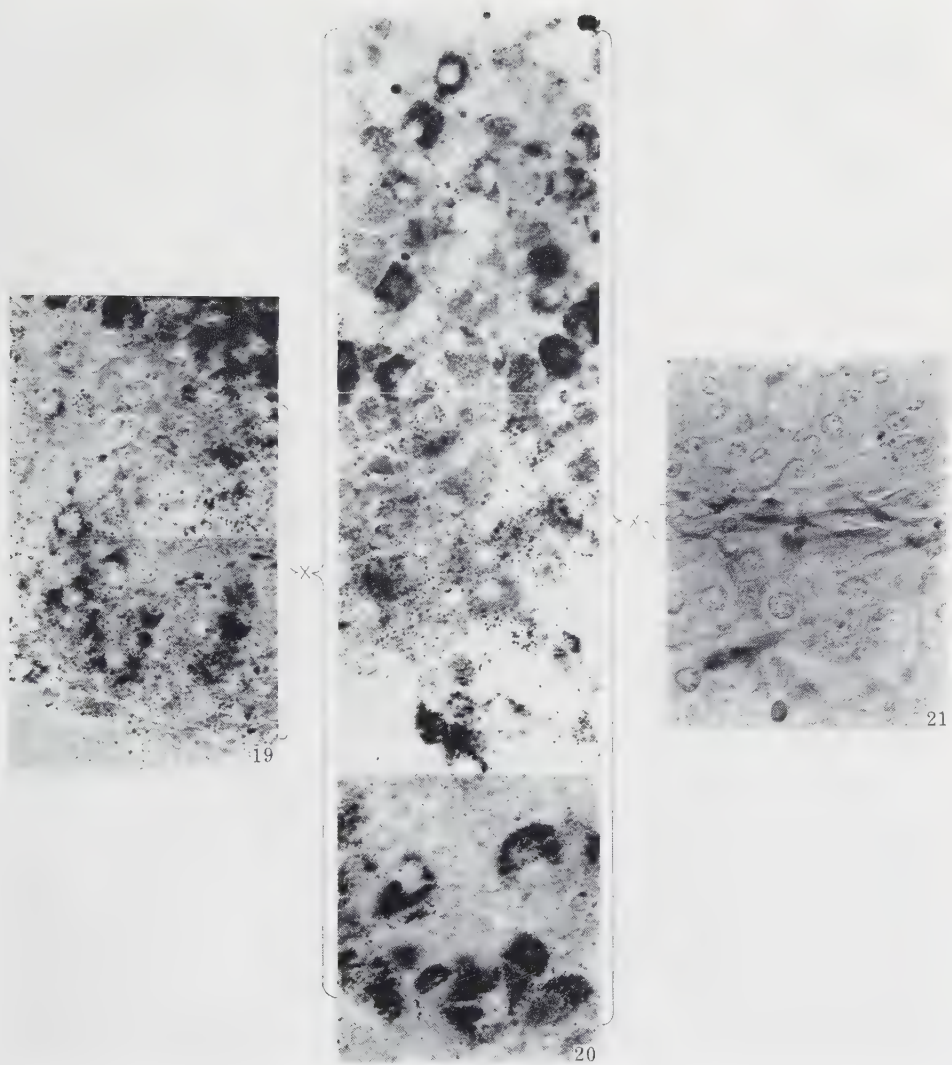


PLATE 4

EXPLANATION OF FIGURES

High power photomicrographs of the same sections as in plate 1 to show in detail the distribution in the cells of the X-zone of visible osmium-reducing lipid and of other reduced osmium particles. $\times 475$.

- 19 Same section as in figure 7.
- 20 Same section as in figure 8.
- 21 Same section as in figure 9.



STUDIES ON THE SOMITES OF AMBLYSTOMA PUNCTATUM

IV. REPLACEMENT OF THE SECOND TO FOURTH SOMITES OF STAGE 25
BY THE FIFTH TO EIGHTH FROM STAGE 30; AND THE
REPLACEMENT OF THE FIFTH TO SEVENTH BY
THE SECOND TO FOURTH IN STAGE 25

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TWO TEXT FIGURES AND TWO PLATES (SEVEN FIGURES)

INTRODUCTION

In *Amblystoma punctatum* the geniohyoid and the first three segments of the thoracicohyoid muscles are formed from muscle buds (ventral processes) which grow down in cephalocaudal sequence from the lower borders of somites 2, 3 and 4. The geniohyoid and first segment of the thoracicohyoid are derived from somite 2. Somites 3 and 4 furnish, respectively, the second and third segments of the thoracicohyoid.

To determine whether or not more caudal somites may be successfully substituted for somite 2, with the resultant formation of a geniohyoid muscle in whose development they do not normally participate, Adelman and Maclean ('35) replaced somites 2 to 4 by somites 3 to 5, 4 to 6 or 5 to 7 from donors of the same stage (Harrison stage 25) of development. It was found that when somites 3 to 5 were substituted for 2 to 4, a geniohyoid muscle was formed from somite 3, but that it was usually somewhat more slender than its counterpart of the normal side. The first two segments of the thoracicohyoid were formed from somites 3 and 4, respectively, and were in most cases slightly more slender than normal.

When somites 4 to 6 were substituted for 2 to 4, a geniohyoid was formed from somite 4, but it was characteristically only about one-half as broad as the normal muscle. The first two segments of the thoracohyoid were formed from somites 4 and 5, respectively, and revealed in most cases a slight diminution in size.

A geniohyoid failed to develop when somites 5 to 7 replaced 2 to 4. Somite 5, however, formed the first segment of the thoracohyoid which was markedly reduced in size. The second segment of the thoracohyoid, formed from somite 6, was affected to a lesser degree, while the third segment, derived from somite 7, was practically normal in size.

In interpreting the above results Adelman and Maclean ('35) considered as improbable the possibility that the failure of the geniohyoid to form from somite 5 was due to an inherent inability to form that muscle. The effect of a number of possible factors was considered in evaluating the results: a) a time factor reflecting the cephalo-caudal order of formation of the somites and their ventral processes, b) a quantitative factor due to slight graduated differences in the size of the ventral processes of the somites, c) environmental influences, and d) possible qualitative differences expressed as a decreasing ability to accommodate to a new situation.

In the experiments above described, host and donor being of the same age, somites 2 to 4 were replaced by ontogenetically younger somites whose ventral processes normally form at a slightly later time than those of the somites they replace. The transplanted somites were consequently temporally 'out of step' with their new environment and, hence, at some disadvantage. It is conceivable that this handicap might be great enough to prevent the formation of a geniohyoid from somite 5 when it replaces somite 2.

Adelman and Maclean ('34a) have shown that when somites 1 to 5 from a donor in stage 25 are substituted for the same somites of a host in stage 30, the ventral processes of the grafted somites are retarded as compared with those of the normal side. They also found that the geniohyoid

formed from implanted somite 2 is in most cases more slender than its counterpart of the normal side. A lag in the development of the ventral processes of somite 2 seems, therefore, to affect the size of the geniohyoid in a manner comparable to what has been observed after the substitution of somites 3 or 4 for somite 2. A similar condition was observed after the dorsoventral rotation of somites 2 to 4 (Adelmann and Maclean, '34 b).

The present paper presents the results of an experiment designed to overcome the age handicap apparently suffered by somites 5 to 8 when substituted for somites 2 to 4 in animals of the same age. In the present experiment somites 2 to 4 of hosts in stage 25 were replaced by somites from donors in stage 30.

After this operation a geniohyoid may be formed from somite 5, but it is in almost all cases more slender than the normal muscle. This in spite of the fact that, in the majority of younger animals examined, no appreciable lag was observed in the development of the ventral processes of the grafted somites as compared with those of somites 2 to 4 on the normal side of the host. Indeed, in some cases the ventral processes of grafted somites 5 to 7 were in advance of those from somites 2 to 4 on the normal side. It thus appears that the elimination of the handicap due to developmental age, while making possible the formation of a geniohyoid from somite 5, does not result, in most instances, in the formation of a muscle of normal size in young larvae. There now appears to operate a quantitative factor, possibly a reflection of the fact that the ventral process of somite 5 is normally more slender than those of more cranial somites and hence provides less material.

The second experiment to be reported here consists in the replacement of somites 5 to 7 by somites 2 to 4 from a donor in the same stage (25). Somite 2, which replaces somite 5, now forms the fourth segment of the thoracicohyoid and makes no attempt to form a geniohyoid. The fourth segment of the thoracicohyoid formed from somite 2 is stouter than

its normal counterpart. This result is comparable to that observed by Adelman and Maclean ('35) when somite 2 replaced somite 4 after the reversal of the anteroposterior axes of somites 2 to 4, and is, probably, an indication of the operation of the quantitative factor above referred to. This series provides further evidence of the lack of a mosaic of muscle anlagen.

MATERIAL AND METHODS

In the first series of operations somites 5 to 8 were removed from donors in Harrison's stage 30 and implanted in place of somites 2 to 4 removed from hosts in stage 25. In the second series of operations somites 5 to 7 were removed in stage 25 and replaced by somites 2 to 4 from donors in the same stage. Since the operative procedure has been adequately described in previous papers no further details need be added here.

The specimens were fixed in Bouin's fluid, stained in toto with Mayer's HCl carmine, and sectioned at 10 μ . A rigorous selection of the sectioned material was made and all animals in which the operation did not appear to have been completely successful were eliminated.

EXPERIMENTAL

Series I. Host state 25. Donor stage 30. Somites 2 to 4 replaced by somites 5 to 8. Orientation normal

For descriptive purposes the material has been divided into two age groups. The ten specimens of the first group ranged from stage 34 to 39 at the time of fixation. In no case had the geniohyoid muscle begun to form on the normal side, but the ventral muscle buds were in most instances still connected with their somites of origin.

In only one case (Amb. 41, 1935; stage 38) were the ventral muscle buds of the grafted somites slightly retarded as compared with the normal side, and in this instance the down-growth from somite 5 failed to develop.

In five specimens the downgrowths from the grafted somites had advanced ventrally about as far as those from somites 2 to 4 on the normal side. In two of these animals, however, implanted somite 8 failed to form a ventral process, its downgrowth apparently having been blocked by the pronephros.

The downgrowths from somites 2 to 4 on the normal side of one of these five animals (Amb. 35, 1935; stage 34-35) may be compared with those from somites 5 to 8 on the operated side in figures 3 and 4. While the ventral extent of the muscle buds is approximately equal on both sides, those on the operated side appear to be slightly in advance histologically since under the microscope the cells appear to be noticeably freer of yolk granules.

Figures 1a and 1b are reconstructions ($\times 40$) of the normal and operated sides of an older specimen (Amb. 40, 1935; stage 39). The ventral processes from grafted somites 5 to 7 have attained the same level as those from somites 2 to 4 on the normal side, but are still connected with their somites of origin. The ventral process of grafted somite 8 has failed to develop.

In the remaining four specimens the downgrowths from somites 5 to 7 or 8 are definitely in advance of those from somites 2 to 4 on the normal side. Reconstructions of the normal and operated sides of one of these cases (Amb. 38, 1935; stage 34) are illustrated in figures 2a and 2b. Another specimen (Amb. 44, stage 38) is especially interesting because the geniohyoid has already begun to grow forward from the first segment of the thoracicohyoid on the operated side, extending forward for a short distance immediately lateral to the thyroid anlage (fig. 5). On the normal side the first segment of the thoracicohyoid has extended ventrally to approximately the same point but is distinctly less advanced histologically and the geniohyoid has not yet begun to grow forward from it. A similar result was observed when somites 2 to 4 from a donor in stage 30 were substituted for the same somites in a host in stage 25 (Adelmann and Maclean, '34 a).

The first group of ten younger specimens thus demonstrates clearly. 1) that ventral muscle buds or processes originate from the grafted somites, 2) that the transplantation of somites 5 to 8 from stage 30 has in 90% of all cases eliminated and in 40% more than compensated for the time handicap which results when these somites from donors in stage

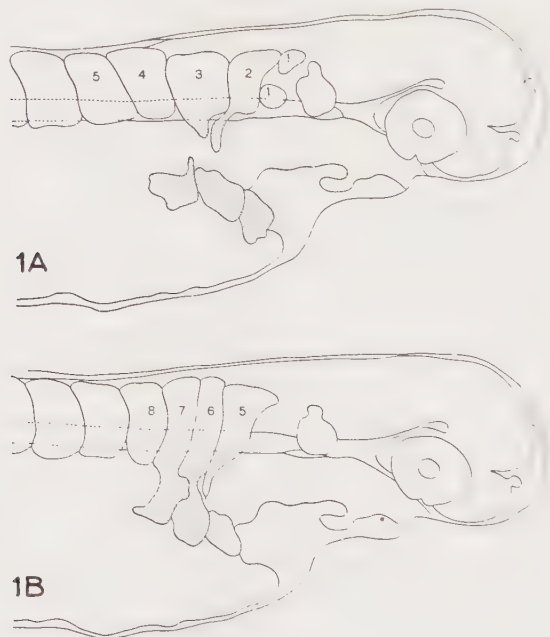


Fig.1 Reconstructions ($\times 25$) of the normal (1a) and operated (1b) sides of *Amblystoma* 40, 1935, fixed in stage 39. On the operated side the ventral processes of grafted somites 5 to 7 have grown down to approximately the same level as those from somites 2 to 4 on the normal side, but they are still connected with their somites of origin. The ventral process of grafted somite 8 has failed to develop. The ventral processes of the somites are stippled.

25 are implanted at the side of somites 2 to 4 in animals of the same age, and 3) that in some cases the downgrowths from somites 5 to 8 may actually be in advance of those originating from somites 2 to 4 on the normal side of the younger host.

The second group comprises twenty animals fixed in stage 46 where the geniohyoid is well developed on the normal side in all cases. In eight specimens (40%) no geniohyoid has formed on the operated side. In twelve cases (60%) a geniohyoid has been formed from grafted somite 5. In two of these

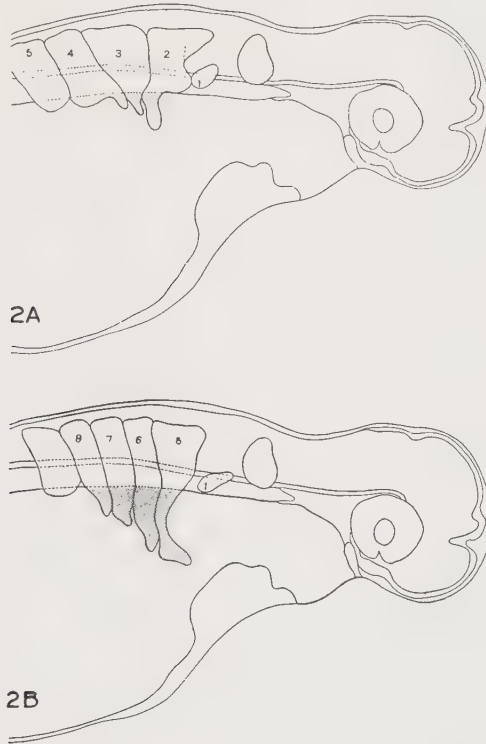


Fig. 2 Reconstruction ($\times 25$) of the normal (2a) and operated (2b) sides of Amblystoma 38, 1935, fixed in stage 34. The downgrowths from somites 5 to 8 on the operated side are definitely in advance of those from somites 2 to 4 on the normal side. The ventral processes of the somites are stippled.

twelve cases the geniohyoid formed from somite 5 is of approximately normal size, but in one of these cases, a slip from the anterior end of the geniohyoid passes over the midline to join the muscle of the normal side. This results in a reduction in the size of the anterior end of the geniohyoid on

the operated side. This condition is perhaps a reflection of the precocity of its development. Normally, when the geniohyoid begins to grow forward below the pharynx, it is separated from its fellow of the opposite side by the thyroid anlage to which it is closely opposed. If, however, the geniohyoid on the operated side grows forward before the geniohyoid of the normal side (*vide supra*) and before the thyroid has attained its definitive caudal extension, it is conceivable that some fibers of the precocious muscle may cross the mid-line and thus produce the condition here described. In the ten remaining animals in which a geniohyoid has formed on the operated side, that muscle is in all instances more slender than its normal counterpart, that is, from one-fifth to three-fourths normal size.

It is difficult to generalize the conditions prevailing in the segment of the ventrolateral musculature derived from grafted somite 8 interposed between segment 3 of the thoracohyoid and the segment formed from somite 5 of the host (segment 4 of the normal series). In accordance with expectations, there is, in fact, clear evidence (fig. 6) in many cases of an additional segment interposed between the third segment of the thoracohyoid derived from implanted somite 7 and the segment derived from somite 5 of the host, the last named being a counterpart of the fourth segment of the ventrolateral musculature on the normal side. The interpolated segment formed from grafted somite 8 shows, when present, a tendency to fuse with either the third segment of the thoracohyoid derived from implanted somite 7, or with the segment formed from somite 5 of the host. The partial fusion of the ventral downgrowths from implanted somites 7 and 8 was observed in a younger specimen in stage 38-39 and is probably to be interpreted as the result of crowding. The absence of an interpolated segment in the thoracohyoid can be explained on the basis of observations made on younger specimens where the formation of a ventral downgrowth from somite 8 was apparently blocked by the pronephros.

*Series II. Host and donor stage 25. Somites 5 to 7
replaced by somites 2 to 4. Orientation normal*

Nine animals sectioned in stages 34 to 37 showed clearly the formation of ventral processes from the grafted somites 2 to 4. Since the grafted somites are slightly older developmentally than the somites which they replace, it was expected that the downgrowths from these grafted somites would show a slight advance as compared with those formed from somites 5 to 7 on the normal side. In some cases, however, this point could not be accurately determined from the study of sagittal sections which were not uniform on the two sides of the body. In one specimen sectioned frontally the ventral downgrowths from grafted somites 2 to 4 are definitely not in advance of those from somites 5 to 7 on the normal side. In most cases, however, there is no doubt that the downgrowths from the grafted somites are slightly in advance. This condition is illustrated in the specimen illustrated in figures 7 and 8 (Amb. 75, 1935) where the ventral processes of grafted somites are conspicuously stouter and extend farther ventrally than those from somites 5 to 7 on the normal side.

In a group of ten older animals in stage 46 segments 4, 5 and 6 of the ventrolateral musculature are essentially normal in morphology on the operated side where they have been formed from the grafted somites. In some specimens these segments are connected with their somites of origin by strands of muscle, and in some cases their appropriate innervation is evident.

These segments derived from the grafted somites appear in general to be slightly stouter than the comparable segments of the normal side. This is particularly true of the fourth segment derived from somite 2. A good illustration of the difference in size which, in the best cases, prevails between this segment on the normal and operated sides of the body is provided in figure 9. The difference is not conspicuous in segments 5 and 6 which are normally very thin in their ventral portions. What has been for convenience termed a quan-

titative factor seems to be expressed here particularly in the case of the segment derived from somite 2. It will be discussed later.

Of particular importance is the fact that in neither the younger nor the older animals of this series was there any evidence of an attempt at the formation of a geniohyoid muscle from somite 2 in its new environment.

DISCUSSION

From the foregoing description, the first point which emerges clearly is the fact that somite 5 is potentially able to form the geniohyoid and the first segment of the thoracicohyoid muscle although in the normal course of events it would have furnished merely the fourth segment of the latter muscle.

It is thus evident that the failure of the geniohyoid to form when somites 5 to 7 replace somites 2 to 4 in animals of the same age was not due to any inherent inability on the part of somite 5 to form the geniohyoid. It was, as Adelmann and Maclean ('35) concluded, probably due to the lag in the development of the ventral processes of the grafted somites. This lag was less marked when somites 3 to 5 or 4 to 6 replaced somites 2 to 4; consequently a geniohyoid was formed from somites 3 and 4 respectively. In the first series of experiments reported in this paper the lag observed when host and donor are the same age was compensated for by the transplantation of somites 5 to 7 from an older donor. The ventral processes of the grafted somites were thus relieved of the disadvantage normally imposed upon them as a result of the cephalo-caudal sequence in the order of their formation. The growth of their ventral processes was better correlated with the developmental processes taking place in their new environment, and the fifth somite was consequently able to form the geniohyoid.

It has, furthermore, been found that the geniohyoid and the first segment of the thoracicohyoid formed from somite 5 are, in most cases, markedly smaller than normal.

A similar reduction in the size of these muscles has previously been reported after the following operations: 1) replacement of somites 1 to 5 of an animal in stage 30 by the same somites from an animal in stage 25, 2) replacement of somites 2 to 4 of an animal in stage 25 by somites 3 to 5 or 4 to 6 from an animal in the same stage and 3) rotation of the dorsoventral axis of somites 2 to 4 in stage 25 (Adelmann and Maclean, '34 a, '34 b, '35). In the first case the reduction in size of the geniohyoid is probably to be attributed to a lag in the development of ventral processes occasioned by age differences between somites 2 to 4 of host and donor. In the third case the reduction in size may be reasonably ascribed to a lag probably due to the time necessary for the inverted somites to adjust themselves to the altered conditions, while in the second case the reduction in size is probably due in part to the lag occasioned by what has been termed a time factor, reflecting the cephalo-caudal order of formation of the somites. Here a quantitative element, now to be discussed, must also be taken into account.

We have seen that when somites 5 to 7 from a donor in stage 30 replaces somites 2 to 4 in a host in stage 25, the ventral processes of the grafted somites were retarded in only one of ten animals studied in suitable stages. It would appear, therefore, that in this case some other factor is responsible for the reduction in size of the geniohyoid and first segment of the thoracicohyoid observed after this operation in ten (83%) of twelve animals in stage 46. This factor has been termed for convenience a quantitative one. The evidence for its operation may now be briefly examined.

It has been observed (see Adelmann and Maclean, '34 a) that the ventral processes of somites 2 to 4 are normally stouter than those of more caudal somites. Their cells are also somewhat richer in yolk granules. This may possibly be interpreted as one of those anticipatory phenomena so generally encountered in the embryo. The second somite, for example, forms in addition to the geniohyoid, the stoutest segment of the thoracicohyoid. The second and third seg-

ments of the thoracicohyoid which are normally formed by the third and fourth somites, while progressively more slender than the first segment, are somewhat bulkier than more caudal segments of the thoracicohyoid. There is thus a progressive decrease in the amount of muscle normally furnished by the ventral processes of these three somites. In the case of somites 2 and 3, particularly, this is considerably in excess of the mass of muscle normally furnished by somites caudal to the fourth. Consequently, it is possible that the progressively smaller geniohyoid muscles formed by the ventral processes of somites 3 or 4 when somites 3 to 5 or 4 to 6 replaces somites 2 to 4 in animals of the same age (Adelmann and Maclean, '35) is due to the operation of at least two factors, 1) a time or age factor reflected in the lag referred to, and 2) a quantitative factor expressive of the sizes of their ventral processes. This may place quantitative limits upon the performances of the individual somites. The operation of both of these factors which becomes progressively more evident when somites 3 to 5, and 4 to 6 replace 2 to 4 results finally in the failure of a geniohyoid to form and in a much reduced first segment of the thoracicohyoid when somites 5 to 7 replace 2 to 4 in an animal of the same stage.

When, however (as in the first series of experiments here described), somites 5 to 7 from a donor in stage 30 replace somites 2 to 4 in a host in stage 25, a geniohyoid of reduced size is also formed. In this case, for reasons already advanced, the reduction in size of the geniohyoid is not likely to be the result of a lag in the development of the ventral processes of the grafted somites. It seems, rather, to be almost entirely the result of the operation of the quantitative factor discussed above. The large size of the fourth segment of the thoracicohyoid formed from somite 2 when somites 5 to 7 are replaced by 2 to 4 in animals of the same age (see p. 163), is also evidence of the operation of this quantitative factor. A similar result was reported by Adelmann and Maclean ('35) after the cephalo-caudal reversal of somites 2 to 4.

What has been termed a quantitative factor may, of course, be due to inherent differences in proliferation rate. Sufficient facts are not available for a decision.

The facts here reported raise the interesting question of the possible existence of environmental factors responsible for the forward growth of the geniohyoid muscle from the material furnished by the second somite or from more caudal somites when they are grafted into the site of somite 2. Something in that particular environment seems, obviously to be responsible since the second somite, when transplanted into the site of the fourth or fifth makes no attempt at the formation of a geniohyoid. Is the geniohyoid drawn forward from the main muscle mass by purely mechanical forces associated with the forward growth of the subpharyngeal region? We know that under certain circumstances the geniohyoid on the operated side may grow forward before its fellow of the normal side (Adelmann and Maclean, '34; Adelmann, this paper, p. 159) and while this fact does not preclude the possibility that its forward growth is due to purely mechanical factors, it does leave room for the interesting possibility that some other environmental forces may be responsible for the forward growth of the geniohyoid. Further speculation, however, would be premature.

Finally, the results here presented, together with those previously reported, make it clear that so far as somites 2 to 5 are concerned there is no evidence of a mosaic of muscle anlagen. The evidence indicates clearly that these somites are potentially equivalent in the stages considered. One is tempted to conclude that they are, in addition, harmoniously equipotential, but such a conclusion would be rash at present. We are still uninformed as to the degree of determination of the dermatome, myotome and sclerotome in stage 25. It would also be rash to attempt to extend these conclusions to more caudal somites since there is a possibility that somites caudal to those tested may behave somewhat differently. In this connection it is well to recall that Butcher ('30) found that in the rat the more caudal somites, as soon as they were

formed, seemed to be in a more advanced stage of development than more anterior ones.

SUMMARY AND CONCLUSIONS

1. When somites 5 to 8 from a donor in stage 30 were substituted for somites 2 to 4 of a host in stage 25, the muscle buds (ventral processes) originating from the lower borders of the implanted somites were not retarded, but in some cases were advanced in their ventral extension as compared with those from somites 2 to 4 on the normal side. This differs from the results obtained by substituting somites 3 to 5, 4 to 6 or 5 to 7 for somites 2 to 4 in hosts of the same age (stage 25).

2. When transplanted from a donor in stage 30 to the site of somite 2 in a host of stage 25, somite 5 gave rise to a geniohyoid and first segment of the thoracohyoid, but these muscles were in most instances markedly smaller than normal.

3. These results indicate that the failure of a geniohyoid to be formed by somite 5 when it replaced somite 2 in a host of the same age (stage 25) was not due to any inherent inability to form that muscle.

4. When somites 2 to 4 from donors in stage 25 were substituted for somites 5 to 7 in hosts of the same age, their ventral processes formed segments 4, 5 and 6 respectively, of the thoracohyoid which are normally derived from somites 5, 6 and 7. When the fourth segment of the thoracohyoid was formed by somite 2, it was usually stouter than its normal counterpart. In its heterotopic position, somite 2 made no attempt to form a geniohyoid muscle.

5. These results are interpreted as due in varying degrees to the operation of a number of factors: 1) differences in age of somites, 2) quantitative differences in the size of the ventral processes of the somites, 3) environmental influences, and possibly, slight qualitative differences.

6. Aside from age and quantitative differences, somites 2 to 5 seem to be potentially equivalent and there appears to be no evidence of a mosaic of muscle anlagen.

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PLATE 1

EXPLANATION OF FIGURES

3 and 4 Retouched photographs ($\times 79$) of sagittal sections of the normal (fig. 3) and operated (fig. 4) sides of *Amblystoma* 35, 1935, fixed in stage 34-35. See text for description. v.p., ventral process.

5 Retouched photograph ($\times 79$) of sagittal section of the operated side of *Amblystoma* 44, 1935, fixed in stage 38. The geniohyoid has already begun to grow forward from the first segment of the thoracicohyoid. On the normal side (not illustrated) it has not yet begun to do so. See text for description. gh.m., anlage of geniohyoid muscle.

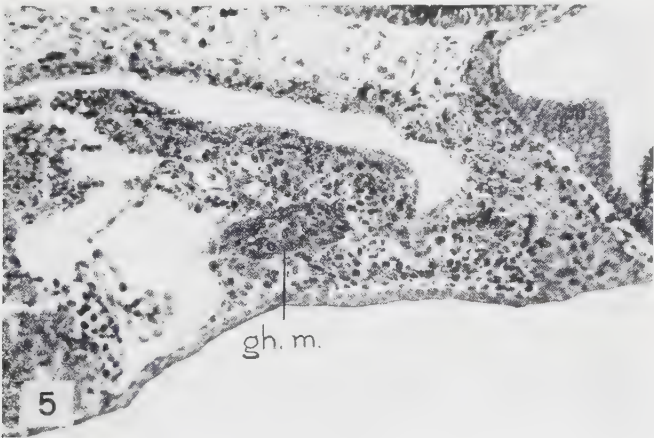
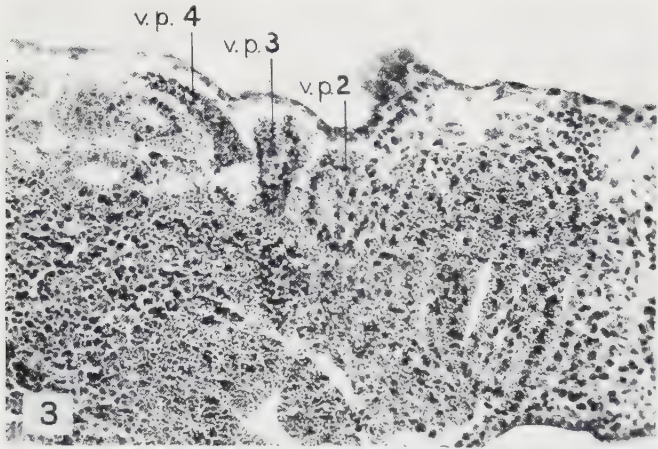


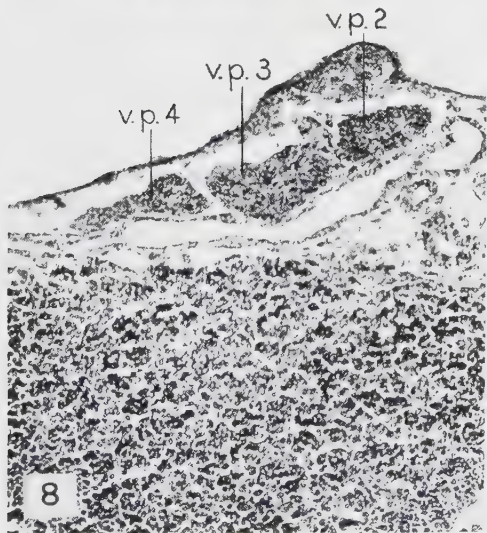
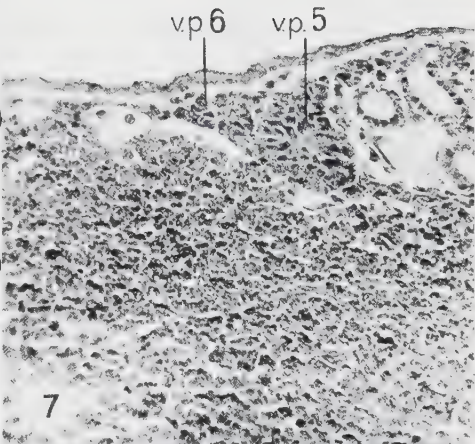
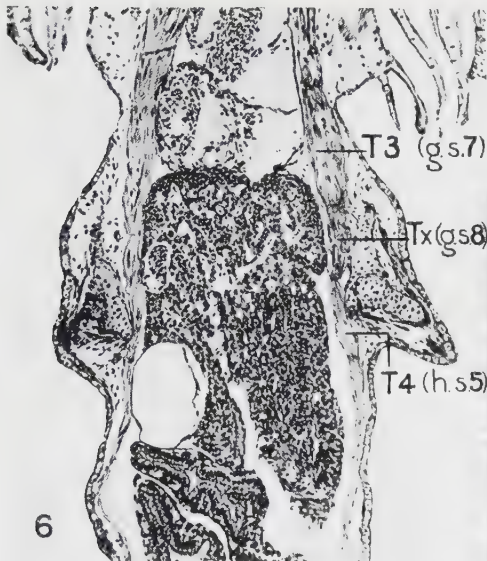
PLATE 2

EXPLANATION OF FIGURES

6 Retouched photograph ($\times 29$) of frontal section of *Amblystoma* 45, 1935; stage 46, to show extra segment (Tx; derived from implanted somite 8) interpolated between the segment 3 of the thoracicohyoid (derived from implanted somite 7) and the segment derived from somite 5 of host. g.s., grafted somite; h.s., host somite; T., segment of thoracicohyoid muscle.

7 and 8 Photographs ($\times 79$) of sagittal sections of normal (fig. 7) and operated (fig. 8) sides of *Amblystoma* 75, 1935. See text for description. v.p., ventral process of somite.

9 Photograph ($\times 38$) of frontal section of *Amblystoma* 80 to show the large size of the fourth segment of the ventrolateral musculature derived from grafted somite 2. g.s., grafted somite; T., segment of thoracicohyoid muscle.



PHYSICAL CONDITIONS IN THE UTERUS GOVERNING THE DURATION OF PREGNANCY ¹

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TWO TEXT FIGURES AND ONE PLATE (FOUR FIGURES)

The conditions governing the duration of pregnancy and its normal termination are usually held to be harmonic in nature. Thus it is generally stated that one hormone exerts a stabilizing effect upon the myometrium and trophoblast while another one contributes to the activation of the uterus at term and so to the onset of labor. While it is important to know the hormone balance throughout the course of pregnancy as well as the conditions which govern this, it is clear that the several hormones exert their effects through changes in the metabolism of the tissues and these, in turn, create physical, vascular, and growth changes in the gravid uterus which prepare it for evacuation. In this paper, data bearing upon the chief physical adjustments that occur during gestation in the rabbit are considered and these in turn are correlated with the principal vascular changes which take place simultaneously. The basis of these experiments is found in recent work of Wishart and Hammond ('33).

These investigators have established, by statistical treatment of data from genetically pure strains of rabbits, that the greater the number of fetuses in a litter, the shorter (within limits) will be the duration of pregnancy. Conversely, the smaller the litter-size, the longer does pregnancy last. At the same time, it is also demonstrated that the larger the litter

¹Supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

size, the greater is the total mass of conceptus tissue and fluids. It accordingly follows that the greater the distention-mass at the end of pregnancy, the shorter will be the duration of pregnancy. This important observation stands as a fact which is valid on statistical grounds. Its physiological basis, however, has not been demonstrated. The following experiments supply this information.

MATERIALS AND METHODS

The data listed in tables 1 and 2 were obtained from twenty rabbits of mixed stock on selected days of pregnancy. The rabbits were mated as soon as possible after a period of isolation, and were killed on different days, some on the sixteenth, some on the twenty-second and still others on the twenty-eighth or thirty-first day of pregnancy. The day of mating was counted as the first day of gestation.

At the time of killing, each rabbit was anesthetized with Dial (Ciba) anesthesia and a small incision, usually about 1 cm. in length, was made in the lateral abdominal wall, as nearly as possible over a distended ampulla of the gravid uterus. It was seldom necessary to disturb the viscera to insert a small (no. 25) hypodermic needle into the region of the center of the sac of fluid. Intrauterine pressure was then determined by measuring the amount of pressure which was just necessary to initiate movement (0.1 to 0.3 mm.) toward the uterus of a bubble of air in a narrow column of water. The pressure in the system was then lowered, the bubble returned to its original position and the pressure determination made again. This procedure was repeated fifteen to twenty times in the course of 10 to 20 minutes, and the mean pressure calculated. Since the uterus may contract at irregular intervals during pregnancy, the pressures in any series varied somewhat. The mean pressures noted in table 2 are accompanied, therefore, by the standard deviation in each case. In making the pressure readings it was found that similar values were obtained regardless of whether the abdominal incision was small or large.

TABLE 1
Weight of conceptus and its parts (including maternal placenta)

DAY OF PREGNANCY AND RABBIT	TOTAL NUMBER FETUSES	FETUS		MATERNAL PLACENTA		FETAL PLACENTA		FETAL FLUIDS		CONCEPTUS WEIGHT		WEIGHT OF PRODUCTS OF CONCEPTION ¹		WEIGHT OF UTERUS ²		DISTENTION COEFFICIENT
		gm.		gm.		gm.		cu.cm.		gm.		gm.		gm.		
16th-20	10	0.37		1.84		1.03		4.11		7.35		43.1		14.3		3.01
-31	8	1.07		1.43		1.39		2.48		5.37		32.2		15.3		2.10
Average	9	0.72		1.63		1.21		3.29		6.36		37.7		14.8		2.56
22nd-1	5	6.27		1.52		3.85		6.27		17.91		53.7		21.6		2.52
-3	8	4.08		1.93		2.26		3.70		11.97		47.7		16.2		2.94
-2	12	4.58		2.58		2.50		5.01		14.67		119.3		24.3		4.91
-7	12	4.00		1.41		1.59		3.19		10.10		90.9		24.4		3.72
-28	6	4.15		2.35		2.06		3.13		11.69		58.6		20.0		2.93
-27	5	3.90		2.31		2.58		3.96		12.75		42.8		13.5		3.18
Average	8	4.49		2.02		2.47		4.21		13.18		68.83		20.0		3.37
28th-6	7	26.23		2.33		3.59		3.91		36.06		216.4		29.5		7.34
-8	4	27.90		3.31		3.14		3.71		38.06		76.1		24.4		3.12
-10	9	29.14		2.23		3.56		2.00		36.93		184.6		32.55		5.67
-17	7	31.03		1.62		3.90		2.50		39.05		156.2		29.2		5.35
-18	3	36.45		2.40		5.01		4.41		48.27		96.5		26.8		3.60
-19	6	31.65		1.78		3.92		5.91		43.26		173.0		27.6		6.28
Average	6	30.40		2.28		3.85		3.74		40.44		150.5		28.3		5.27
31st-4	8	41.44		2.16		3.42		3.46		50.48		201.9		28.3		7.12
-5	10	45.13		3.13		3.59		0.66		52.51		252.5		28.5		8.86
-23	8	52.34		2.20		3.93		0.67		59.24		236.9		29.2		8.12
-22	8	28.33		1.90		2.86		0.70		43.79		175.1		19.2		9.12
-25	4	58.11		1.95		5.29		1.32		66.67		199.0		16.8		11.84
-30	8	24.65		1.50		2.87		0.84		29.86		119.4		20.8		5.01
Average	7.7	41.66		2.14		3.66		1.27		50.42		197.5		23.8		8.45

¹ In one uterine horn.

TABLE 2

DAY OF PREGNANCY AND RABBIT	SEMI-AXIS		INTRAUTERINE PRESSURES ¹			TENSIONS ON UTERINE WALL ¹			FORMULAE USED IN CALCULATION OF TENSIONS ON UTERINE WALL: SEE TEXT FOR DISCUSSION
	(a) Lateral	(b) Mesial	Observed (p)	Anti- mesial (p + b)	Mesial (p - b)	Lateral	Anti-mesial ¹	Mesial	
16th-20 -31	1.15 1.18	1.26 1.34	1.76±0.32 1.44±0.86	3.03 2.78	0.50 0.10	1.22 1.09	1.90 1.45	0.20 0.52	For days 16 and 22, the shape of the uterus is spheroidal, and frankly elliptical in cross section. Lateral tension: $T = \left(\frac{b^2}{a} \times p \right) \frac{1}{2}$
Average	1.17	1.30	1.60	2.91	0.30	1.15	1.68	0.36	
22nd- -3	1.62 1.35	2.12 1.83	2.00±0.32 2.29±0.81	4.12 4.12	0 0.46	2.78 2.84	2.98 2.05	0 0.23	Antimesial tension: $T = \frac{a^2}{b} \times (p + b) \frac{1}{2}$
-2	1.41	1.78	2.96±0.48	4.74	1.18	3.33	2.64	0.94	
-7	1.35	1.57	2.56±0.75	4.23	0.89	2.64	2.31	0.43	Mesial tension: $T = \frac{a^2}{b} \times (p - b) \frac{1}{2}$
-28	1.37	1.72	4.70±0.98	6.42	2.98	5.08	3.49	1.63	
-27	1.18	1.66	3.85±1.45	5.51	2.19	4.49	2.31	0.92	
Average	1.38	1.79	3.06	4.86	1.28	3.53	2.63	0.69	
28th- -8	1.63 1.66	2.15 1.95	1.55±0.38 1.30±0.36	3.70 3.25	ea. 0 ea. 0	4.38 3.76	4.56 4.58	Ca. 0 Ca. 0	On day 28, the conceptuses are cylindrical, but frankly elliptical in cross section. Lateral tension: $T = \frac{b^2}{a} \times p$
-10	1.63	2.32	2.09±0.93	4.41	ea. 0	6.70	4.36	Ca. 0	
-17	1.72	2.13	1.40±0.08	3.53	ea. 0	3.70	4.92	Ca. 0	Antimesial tension: $T = \frac{a^2}{b} \times (p + b)$
-18	1.83	2.15	1.74±0.45	3.89	ea. 0	4.40	5.98	Ca. 0	
-19	1.65	1.93	1.21±0.41	3.14	ea. 0	3.93	4.44	Ca. 0	Mesial tension: $T = \frac{a^2}{b} \times (p - b)$
Average	1.69	2.10	1.55	3.65	ea. 0	4.32	4.98	Ca. 0	
31st- -4	1.73	1.98	3.78±0.66	5.76	3.78	3.78	5.76	3.78	On the thirty-first day, nearly all fetal fluid is gone (see table 1) and the fetus and membranes lie virtually next to the uterine wall. Consequently, the observed pres- sure is the same over the whole surface of the uterus except for the lowermost part which supports the weight of the conceptus. The values for antimesial pressure and tensions are, therefore, only approximate.
-5	1.70	2.09	4.64±0.54	6.61	4.61	4.61	6.61	4.61	
-23	1.73	2.07	4.21±0.91	6.27	4.21	4.21	6.27	4.21	
-22	1.43	1.84	4.38±1.16	6.23	4.38	4.38	6.23	4.39	
-25	1.63	2.15	2.28±0.26	4.43	2.28	2.28	4.43	2.28	
-30	1.23	1.74	3.95±0.60	5.69	3.95	3.95	5.69	3.95	
Average	1.59	2.13	3.87	6.00	3.87	3.87	6.00	3.87	

¹ cm. H₂O.

Mention should be made of the fact that by the thirty-first day of pregnancy, the uterus is filled almost entirely by the fetus, placenta and membranes, since the fluid undergoes abrupt reduction in amount. Consequently, when the needle was inserted just under the uterine wall, the pressure was found to be about uniform over the whole surface of the ampulla. The significance of this fact will be considered below.

Upon completion of the pressure determinations the abdomen was opened, the uterus bared, and measurements of its shape and size were made with calipers. The measurements included the mesial axis and the lateral axis of each ampulla (see table 2). Afterward, one horn, (the products of conception still contained within it) was fixed in 8% formaldehyde, and prepared for histological study. This included measurement of the average thickness of the uterine wall from planimeter measurements of camera lucida drawings, and study of the arrangement of the musculature. These observations will be reported at a later time. The remaining uterine cornu, the fetuses, and each part of the conceptus (plus the maternal placenta) was weighed. The average of these for each rabbit is listed in table 1.

RESULTS

Growth of the uterus and products of conception. The uterus (one cornu) grows during pregnancy from an organ weighing 1 to several grams to one weighing 20 to 30 gm. By the sixteenth day of pregnancy the uterus is over half its ultimate size and by the twenty-eighth day it is as large as it will be in the course of gestation. There is, accordingly, an initial period of growth which is nearly complete by the twenty-second day and is complete in the next 6 days, well before the time of expected parturition. Data by Hammond ('35) show this better than the present data, and they add the important fact that the period of uterine enlargement begins after implantation of the blastocysts and their initially rapid rate of growth. Hammond likewise shows that the number of

fetuses in a uterus determines in large measure the extent of uterine growth taking place during pregnancy. The reasons for the limited period of uterine growth at the end of pregnancy have been discussed in several other places and need not be reviewed here (Reynolds, '37 a; '37 b; '39).

The conceptuses (plus the maternal placenta), it will be seen in table 1, double their weight between the sixteenth day and the twenty-second day, and by the twenty-eighth day there is more than a six-fold increase in mass of the tissues and fluids distending the uterus. By the thirty-first day, there is more than an eight-fold increase in conceptus-mass over that of the sixteenth day of pregnancy. This total enlargement of the conceptus (plus the maternal placenta), however, is not uniform growth by all its parts. Indeed, for certain parts, it is quite the contrary since the fetal fluids diminish by about 75% in the last 9 or 10 days of pregnancy. The maternal placenta, it will also be observed, shows no increase of mass in the same interval of time while the fetal placenta does not increase in size between the twenty-eighth and the thirtieth days of pregnancy. The latter follows in its growth the same form as does that of the uterus itself. Hammond's data show similar relationships except for the maternal placenta which, in his series of observations, is largest on the sixteenth day and becomes slightly smaller as pregnancy advances. It is evident, therefore, that the factor contributing most to the enlargement of the conceptus is the increasing mass of the fetus, the weight of which on the sixteenth day of pregnancy is about one one-hundredth the weight of a newborn rabbit. Most of this enlargement takes place in the last week of pregnancy at which time the uterus has stopped growing. The adjustments which take place in the uterus at this time, are accordingly, physical ones which involve, among other things, an alteration in the shape of the uterus and in the surface-to-volume (i.e., uterine mass-to-conceptus mass) relationships as pregnancy nears term. Attention was turned, therefore, to these conditions in the gravid uterus throughout pregnancy.

Shape of the uterus in pregnancy. Soon after implantation, the conceptuses (and the maternal placentas) begin to grow rapidly, at the same time distending the uterus locally. At first, the uterus as a whole shows little change from the tubular shape except for the local enlargements at each distention site. In general, this condition persists until about the twenty-second day of pregnancy. The most noticeable change, as shown in plate 1 and in table 2, is an increase in transverse diameter of each enlargement, the shape of which is elliptical at its largest point (fig. 1). Until the twenty-eighth day, both long and short axes show a gradual increase

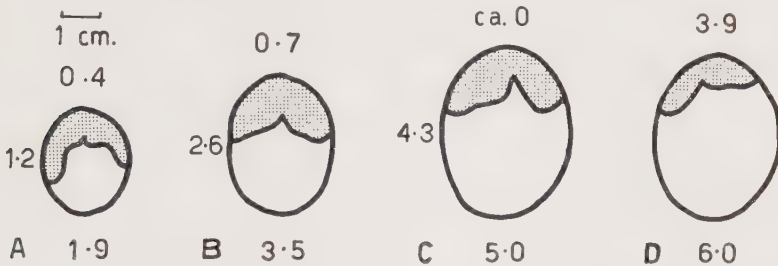


Fig. 1 Diagrams constructed from the data of table 2 showing the maximal cross-sectional diameter of the uterus at the site of a conceptus on the sixteenth (A), twenty-second (B), twenty-eighth (C) and thirty first (D) days of pregnancy. Careful attention was paid to depict the true radius of curvature in each case. The numbers indicate the approximate tensions (centimeters of water) on the mesial wall (top), the lateral walls and the antimesial wall (bottom) in each instance. The stippled area shows the position and extent of the placenta (maternal and fetal) at each stage of pregnancy.

in length, but careful measurements from this day until term show that the mesial-antimesial axis of the distention site does not increase. At the time, therefore, when the uterus has ceased to increase in cross sectional area, it contains a bolus which is growing at its most rapid rate, without increase in transverse diameter. Consequently, the products of conception are accommodated by lengthening of the uterus and the increasing distention associated with this will have a pronounced effect upon intrauterine pressure and the tension

upon the uterine wall. The following data bear out this conclusion.

Intrauterine pressure changes in pregnancy. The changes which take place in intrauterine pressure are noted in table 2. Here, it will be seen, the pressure level nearly doubles between the sixteenth and the twenty-second days. As noted above, enlargement of each conceptus takes place at this time with retention of the spheroid shape. Between the twenty-second and twenty-eighth days intrauterine pressure decreases by some 50%, or to a level similar to that which existed earlier, on the sixteenth day of gestation. This occurs, it will be observed, soon after the time when the uterus virtually ceases to grow and the conceptus enters upon its period of most rapid growth. The fall in pressure is only temporary, however, since by the thirty-first day the pressure is higher than at any previous time in pregnancy, so far as these data go. The apparent paradox of a decrease of intrauterine pressure on the twenty-eighth day at a time of increasing uterine distention has experimental observation as its basis, but it requires explanation. This is supplied by the following considerations.

Surface: volume relationships in the uterus. The intrauterine pressure at any given time is the result of overfilling of the uterine lumen so that the uterine wall resists further stretching. The physical laws for this condition have been stated by Osborne ('09) and by Sutherland ('09) in the case of the surviving bladder. In general, it may be said that intrauterine pressure increases when the tension on the wall increases, and this is a function of the radius (r) of the conceptus-mass. Physiological factors exert their effect upon these pressure and tension relationships through alteration in the rate of uterine growth and through alteration in the tone of the myometrium by changing hormonal conditions as pregnancy comes to an end. Even so, at any given time the tension-pressure relationships hold true, and these are capable of fairly exact expression, provided the shape of the uterus be known.

We have seen that until the twenty-second day of pregnancy the distended ampulla at each conceptus site is spheroidal, although elliptical in cross section (fig. 1), but by the twenty-eighth day, each ampulla approximates a cylinder, although it is still frankly elliptical in cross section. In changing shape from a spheroid to a cylinder, the surface-to-volume ratio undergoes a change such that the pressure which is required to distend the uterus is less than that needed to distend a spheroid of similar diameter. This is exemplified by the simple conditions pertaining to spheres and cylinders respectively. For a sphere, this relationship is

$$\frac{p}{2} = \frac{T}{r}$$

where T is the tension on the wall, r , the radius of the distention-mass and p , the pressure within the sphere. For tube-shaped organs, the relationship is

$$p = \frac{T}{r}$$

The conditions observed in the gravid uteri differ from the above chiefly in that the radius must be reckoned as the semi-axis of an ellipse. This allowance has been made in calculating the data of table 2, and the formula applying in each case is given in the right-hand column of the table. For a brief account of the steps involved in the derivation of the above relationships, the reader is referred elsewhere (Reynolds, '39).²

There is one further consideration which must be taken into account in the present connection. Inasmuch as the intra-uterine pressure is very low throughout most of pregnancy, allowance must be made for the effect of hydrostatic pressure on the tension in different parts of the uterine wall. The antimesial pressure, for example, is taken as the observed intrauterine pressure (measured in centimeters of water near the middle of the sac of fluid) plus one-half the hydrostatic

² The writers are indebted to Dr. H. A. Blair for assistance in applying these relationships to the conditions present in this work.

pressure in the mesial-antimesial axis. Conversely, the pressure on the mesial uterine wall when this is uppermost (e.g., with the rabbit in the upright posture) is the observed intra-uterine pressure less half the hydrostatic pressure in this same axis.

Similar allowances have been made in the calculation of the tensions on the uterine wall. The result is shown in figure 1, in which the mesial, lateral and antimesial wall tensions are given for the gravid uterus on selected days of pregnancy when the mesial margin of the uterus is uppermost. The essential features of this may be summarized as follows.

Tension on the uterine wall during pregnancy. In both table 2 and figure 1, it will be seen that the lateral and antimesial wall tension increases progressively throughout the course of pregnancy, even at the time of diminished pressure on the twenty-eighth day. The development of tension on the mesial wall is quite different, however. On the twenty-eighth day, the mesial wall tension diminishes sharply, coincident with the decrease in average intrauterine pressure. This change results from the low level of pressure in the uterus at this time, and because the weight of the conceptus is chiefly upon the lateral and antimesial walls, when the mesial attachment is uppermost.

One further observation regarding the tension on the uterine wall remains to be made. In table 2 and in figure 1, it will be seen that not until the very end of pregnancy, or just before, is the tension on the mesial aspect of the uterus about equal to that on the other sides of the uterus. This change, the importance of which will be stressed below, occurs because the uterus has ceased to grow and is rapidly distended without further benefit of an advantageous change in the shape of the products of conception. The intrauterine pressure, in fact, increases at this time to such a point that it largely offsets hydrostatic pressure as an important factor in the development of differential tensions upon the uterine wall. One consequence of these changes appears to be that the

circulation of maternal blood through the uterus is adversely affected, as shown by the following considerations.

Local conditions governing maternal blood flow in the placenta. During pregnancy in the rabbit, the volume flow of blood through the maternal vessels of the placenta increases from about 5 cc. per minute on the fourteenth day to a maximum of about 30 cm. on the twenty-second day, whereupon there follows a diminution of about one-third (to less than 20 cc. per minute) by the twenty-fifth day. For a reason unsuspected until now (see below) there occurs a secondary rise by the twenty-seventh day to the maximal rate of flow (Barcroft et al., '33). No data are available which bear upon the rate of flow after this time since technical difficulties preclude such determinations, although Barcroft and Rothschild ('32) have observed that between the twenty-eighth and the thirtieth days of pregnancy a reduction of 50% in the volume of the maternal blood in the uterus takes place.

When these local circulatory changes are correlated with the uterine growth and intrauterine pressure changes (or with the tension on the mesial wall) taking place at the same time, several striking relationships stand out (fig. 2). In the first place, the diminution in the rate of blood flow after the twenty-second day coincides with the onset of a very much reduced rate of uterine growth, before the change in the shape of the conceptus is accomplished. There follows by the twenty-eighth day, however, the change in shape and decrease in intrauterine pressure described above. This coincides precisely with the secondary increase in the rate of blood-flow through the maternal vessels of the placenta. At the time, therefore, when a change of shape in the products of conception brings about a diminution of intrauterine pressure, an obstacle to the flow of blood through the maternal vessels of the placenta is removed. It follows, accordingly, that the local determiner of the efficiency of the maternal circulation in the uterus at the end of gestation is the pressure of the uterine contents upon the placental vascular bed. In the ordinary course of events, the anatomical relationship of the placenta to the rest of

the uterine contents appears to be such that it occupies a position which is favorable to an efficient flow of blood, and so providing an adequate source of nutrition for the growing fetus. This condition lasts only so long, however, as the uterine contents do not distend the uterus to the point that

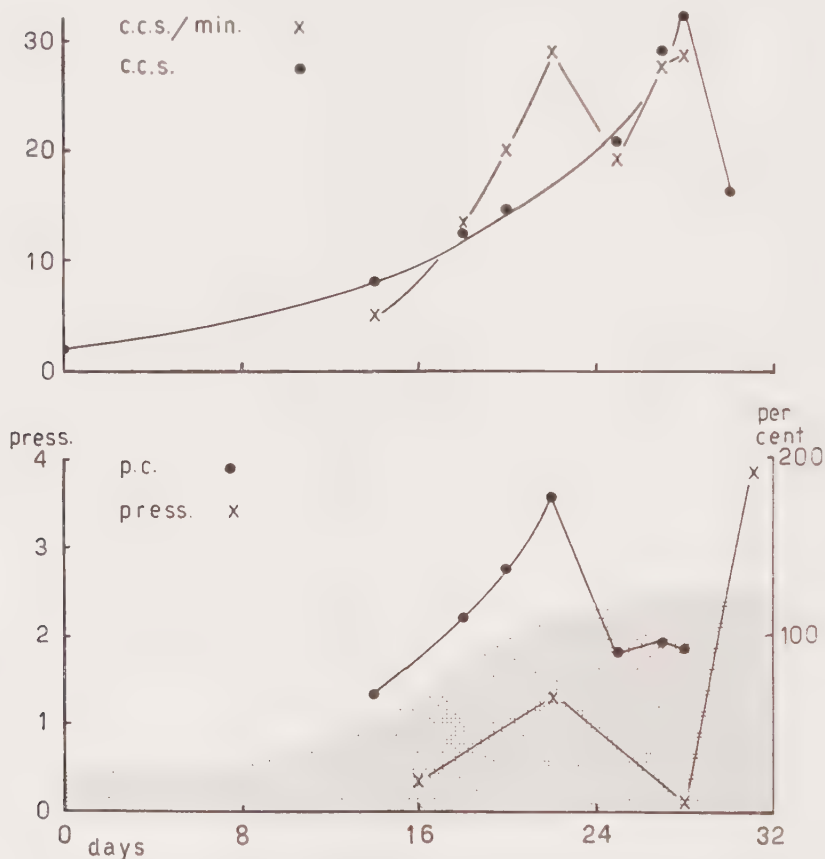


Fig. 2 Correlation graphs showing, above, the rate of blood-flow through, and the volume of maternal blood in the uterus at different times of pregnancy, and below, the course of intrauterine pressure on the 4 days in which it was measured in this work (crosses) and the efficiency of blood flow through the maternal blood vessels in the uterus (dots) estimated as the percentage turnover of maternal blood per minute. Data on blood flow from Barcroft and his associates. The form and relative extent of uterine growth during pregnancy in the rabbit is indicated by the stippled area.

the tension on its mesial wall is about equal to the antimesial or even the lateral uterine wall tension. This occurs at the end of pregnancy, not as the uterus first stops growing, but after the tube-shaped uterus becomes distended to the limit of its capacity to hold the products of conception.

It is necessary to conclude, therefore, that pregnancy comes to an end about the time that pressure on the placenta is approximately equal to the tension throughout the remainder of the uterine wall, and so contributes to the embarrassment which takes place in the flow of maternal blood through the uterus, as Barcroft and Rothschild found between the twenty-eighth and thirtieth days of gestation. This decline in maternal blood volume in the uterus parallels the terminal rise of intrauterine pressure (fig. 2). Unless, therefore, the uterus empties itself of its contents in the normal course of events by the mechanisms of labor, the fetus will perish through its accelerating encroachment upon its own source of nutrition. This is apparently what happens in pregnancies prolonged by exhibition of progesterone near the end of gestation.

Tension on the uterus related to distention. As noted at the outset, Wishart and Hammond established that the larger the degree of uterine distention, the earlier does pregnancy come to an end. Hence it becomes of interest to correlate the tensions on the uterine wall during gestation with the degree of uterine distention.

An index to the degree of distention may be obtained by determining the ratio of the size of the products of conception to the size of the uterus. This quotient, the distention coefficient, is calculated for each of the cases listed in table 1, and is given in the right hand column.

The extent of correlation between this coefficient and the different uterine tensions is shown in table 3. In table 3a, the distention coefficient is correlated with the tension on the mesial wall; in table 3b, the lateral wall tension is correlated with the distention coefficient and in table 3c, the latter value is correlated with the maximal, or antimesial, wall tension in

each case studied. The average values for each of the four groups studied in this series fall in the position indicated in these tables by bold-face type in the enclosures.

From these tables, it is clear that there is no correlation between the degree of uterine distention and mesial wall tension; there is poor correlation between uterine distention and

TABLE 3

*Correlation of uterine wall tension with the degree of uterine distention
(see text for discussion)*

		2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	7-7.9	8-8.9	9-9.9	10-10.9	11-11.9
a. Mesial											
Uterine wall tensions	0-0.9	5	6	1	3	1	1				
	1-1.9	1									
	2-2.9										1
	3-3.9				1		1	1			
	4-4.9							2	1		
b. Lateral											
Uterine wall tensions	1-1.9	2	1								
	2-2.9	2	1								1
	3-3.9		2	1	2		1	1			
	4-4.9		2		1	1	1	2	1		
	5-5.9		1								
	6-6.9			1							
c. Antimesial											
Uterine wall tensions	1-1.9	2	1								
	2-2.9	2	3	1							
	3-3.9	1									
	4-4.9		1		3	1	1				1
	5-5.9		1		1		1				
	6-6.9							3	1		
Distention coefficients											

lateral wall tension, while in each of the four groups studied the tension on the antimesial wall bears a high degree of correlation with the degree of uterine distention.

Inasmuch as Wishart and Hammond have established the relationship between the degree of uterine distention and the duration of pregnancy, and since the present work shows that a high degree of correlation exists between the degree of

uterine distention and the maximal tension on the uterine wall, it is interesting to note that pregnancy in the rabbit comes to an end at the time when the maximal uterine tension becomes about uniform over the surface of the whole organ. This appears to be, accordingly, the physical condition at the end of gestation which signals impending parturition. It comes about as the result of mechanisms of growth-limitation of the uterus, change in shape of the products of conception, and increasing uterine distention, as described above. Whatever other aspects of the labor mechanism this condition may be associated with, it is clear that one important consequence of it is impairment of the nutritional supply of the fetus, and it must come, in the normal course of events, before the myometrium is stretched beyond its capacity to do the work which is demanded of the uterus in labor. The hormonal factors which seem to be concerned in the development of this crucial physical condition for parturition are discussed in some detail in other places (Reynolds, '37 a; '37 b; '39).

SUMMARY

1. Observations are recorded of the rate of growth of the uterus and the parts of the products of conception on the sixteenth day, the twenty-second, twenty-eighth and the thirty-first days of pregnancy in the rabbit.

2. Measurements of intrauterine pressure were made on these days, and from these data, along with data on the shape and dimensions of the gravid uterus in situ, the tensions on different aspects of the uterine wall were calculated.

3. The results show that intrauterine pressure reaches two maximal peaks, the first about the twenty-second day and the second, at term. The basis of the temporary decrease about the twenty-eighth day lies in the change of shape of the conceptus from a sphere to a cylinder, with a concomitant change in the pressure: tension relationships.

4. Associated with the attainment of the first peak of intrauterine pressure is a temporary fall in the flow of maternal blood through the placenta, while at the time of the transient

decrease of pressure a secondary rise in the rate of blood flow takes place through the maternal vessels in the placenta. At the time of the final rapid increase of intrauterine pressure, a marked diminution takes place in the volume of maternal blood in the uterus (Barcroft and Rothschild). The intrauterine pressure is the local determiner of the efficiency of the flow of maternal blood through the uterus.

5. The final increase in intrauterine pressure results from complete cessation of uterine growth at the time of most rapid fetal growth.

6. The onset of labor in the rabbit coincides with a degree of uterine distention which imposes upon the mesial wall, i.e., placental vascular bed of the uterus, tension about equal to the maximal tension occurring elsewhere on the uterine wall. Until this time, anatomical and physiological conditions are such that the tension on the mesial wall remains relatively low. Pregnancy comes to an end in the rabbit when the pressure on the placental vascular bed becomes sufficiently high that it affects adversely the efficiency of the flow of maternal blood through this organ, so reducing the nutritional supply at a time when the fetus is growing most rapidly.

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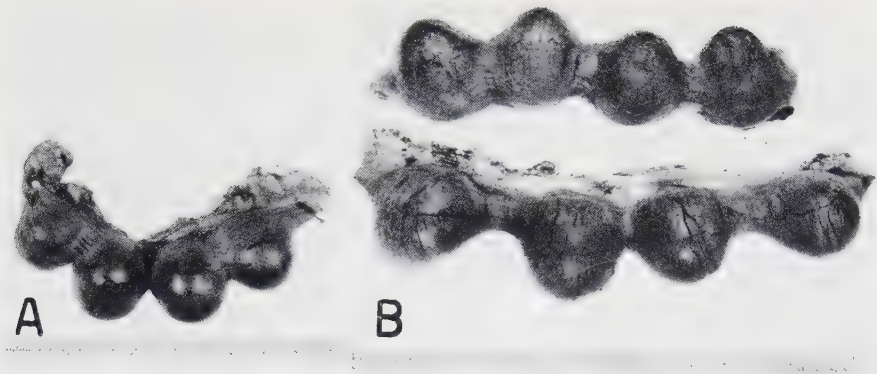
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PLATE

PLATE 1

EXPLANATION OF FIGURES

Uterine cornua containing four conceptuses each, shown in the same scale to indicate the size and shape of each ampulla containing a conceptus on the sixteenth day of pregnancy (A), the twenty-second day of pregnancy (B), the twenty-eighth day of pregnancy (C) and the thirty-first day of pregnancy (D). In B, two uteri are shown, the one above being from a rabbit having twelve conceptuses, the one below from a doe having eight conceptuses. The effect of litter-size on the size of the conceptuses is clearly seen.



FACTORS AFFECTING THE POSTNATAL GROWTH OF THE LUNG

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FOUR FIGURES

These experiments represent the first in a series of studies of the growth of the lung of the rat, and the factors which influence the rate of growth of the lung.

It has been suggested (Walter and Addis, '39) that, after weaning, change in the weight of certain organs may be determined mainly by change in organ work. But the lung does not do work in the same sense as the heart, kidneys, liver, and other glandular organs. Apparently the aeration of the blood is merely a passive function. And yet it is known that under certain conditions, the lung can increase greatly in size and weight. It will be the purpose of this paper to show that this increase is due solely to the mechanical stimulus of the change in the pull exerted by the alteration in size of the thoracic cage which the lung must fill. Consequently, these responses will ordinarily take place only in a growing animal, a qualification possessed by the rat throughout the greater part of its life.

Normal heart and lung weights. For purposes of future comparison the normal heart and lung weights of a series of animals were plotted against the body weight of the animals. The rats used were albino males of the Slonaker strain kept under standard conditions of housing, temperature, and diet. Before weighing the organs, the animals were anaesthetized with ether, the abdomen opened, and the aorta cut.

After bleeding had stopped, the thoracic viscera were removed, trimmed and blotted. The heart was opened and blotted before weighing.

From the graph in figure 1 it is apparent that, within the range of body weight used, the total lung weight, or any constant anatomical subdivision of the total lung weight, may be considered to bear a straight line relationship to the body weight.

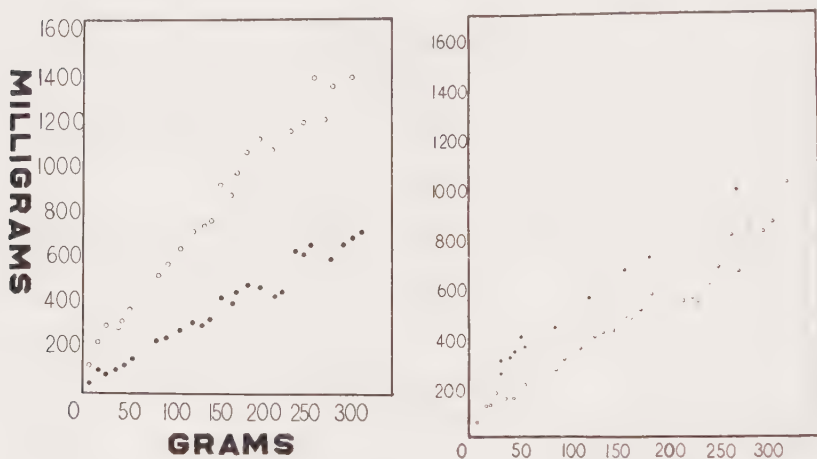


Fig. 1 Total lung weights in milligrams (clear circles) and left lung weight in milligrams (black circles) plotted against the body weights in grams. Each point represents an average of several rats of similar body weight. The actual data are given in table 1.

Fig. 2 Right lung weights of the normal rat (clear circles) compared to the right lung weights of rats which have had left upper lobectomy 14 days previously (black circles).

Older animals were not used because of the frequent presence of lung disease. These findings are in agreement with similar data presented by Hatai ('13) and Jackson ('13) for rats between the body weight of 50 gm. and 350 gm. Male organ weights are slightly higher than female organ weights.

The effect of lobectomy. Since the left upper lobe is the largest single lobe, this was selected for removal. Under ether anaesthesia, a low left intercostal incision was made.

The periphery of the lobe was gently grasped and pulled through the incision. A silk ligature was rapidly placed around the hilum, the lung cut off, and the mediastinum allowed to fall back. The chest wall was then closed. The

TABLE 1
Normal controls

<i>Number rats</i>	<i>Range in body weight gm.</i>	<i>Average heart weight mg.</i>	<i>Average weight right lung mg.</i>	<i>Average weight left lung mg.</i>
6	360-390	1067	962	682
13	310-317	930	934	693
10	300-308	890	850	666
14	290-309	865	810	640
8	280-290	878	852	600
12	270-277	778	668	524
10	260-269	786	708	602
11	250-257	814	675	538
14	240-249	821	625	556
7	230-239	789	562	440
12	220-228	713	573	426
15	210-219	699	555	435
13	200-210	620	550	469
11	190-199	620	597	468
9	180-189	628	594	457
11	170-179	588	544	441
18	160-167	582	503	401
14	150-158	600	509	426
16	140-148	514	425	345
12	130-137	510	416	336
7	120-124	460	415	330
13	110-118	425	360	282
10	90-106	385	317	258
12	80-90	374	270	250
12	50-60	242	216	102
19	40-49	206	169	132
11	30-39	209	164	125
10	20-28	147	158	99
13	10-18	79	132	105
31	5-9	51	59	48

operation must be done rapidly as the rat will not tolerate an open pneumothorax for more than 3 to 4 minutes. Kinking of the mediastinum or tearing of the lung will cause death. The operative mortality was about 10%, being slightly higher in younger rats.

After a period of 14 days, the rats were killed in the same manner as the control group and the heart and lungs weighed. When plotted (fig. 2) these data show that the right lungs in the lobectomized animals are larger than the controls. Furthermore, the amount of this increase in weight of the right lung in the lobectomized animal approximates the weight of the left upper lobe which was removed. It is thus apparent that if a certain amount of lung tissue is removed, its equivalent weight will be replaced, in a period of 14 days, by the remaining lung tissue.

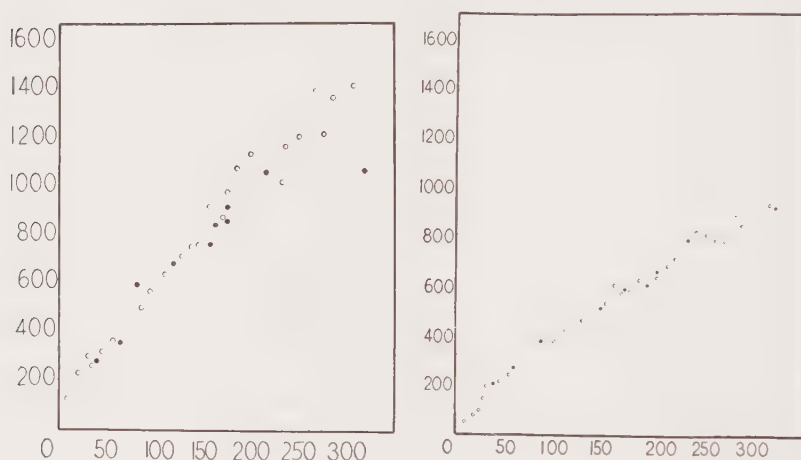


Fig. 3 Total lung weights of the normal rat (clear circles) compared to total lung weights of rats which have had varying amounts of lung tissue removed 14 days previously (black circles).

Fig. 4 Heart weights of the normal rats (clear circles) compared to the heart weights of rats which have had varying amounts of lung tissue removed 14 days previously (black circles).

To determine whether this conclusion might have been vitiated by failure to remove completely an exact anatomical division of the lung, total lung weights after operation were compared to the total lung weights of normal animals, at the end of the 14-day period. Figure 3 shows that the total lung weights of the operated animals were equal to that of the controls. In other words, no matter how much lung tissue was

successively removed, the animals regained the entire lung weight lost at operation.

The rate at which this restitution of lung tissue takes place is modified by the age of the rat. Young rats will restore their total lung weights to the normal within short periods of time whereas the older rats sometimes do not approximate the normal lung weight even 30 days after operation. It was possible in some cases to remove the entire right lung and the left lower lobe. In rats under 250 gm. in body weight, the remaining left upper lobe would equal in weight the five normal lobes of an unoperated rat of similar body weight.

The heart weights, on the other hand, were only slightly heavier than the controls in all the experiments of short duration. After the 30-day period, there was a definite increase in heart weight over the normal. Where only one lobe was left at operation, the heart weight increased as much as 40%.

Table 2 shows the effect of length of time after operation upon change in weight of the heart and lung.

The effect of increasing the size of the thoracic cage. In order to increase the stretch on the lungs by enlarging the mean respiratory volume of the thoracic cage, rats were kept at a pressure of —500 mm. of mercury. Barcroft in his 'Lessons From High Altitudes' showed that among the responses of the body in acclimatizing itself to high altitudes, increased thoracic volume played a part. The Cholos had not only broader chests than the white men but also their ribs were in a more horizontal position as demonstrated by x-rays. The white men not only had to breathe more quickly but also, when moving, had to stop frequently and consciously take several long deep breaths so that the lungs were forcibly stretched more, and more often, than at sea level.

The rats were placed in an air tight iron tank which had two outlets. One outlet was connected to a vacuum pump, the other to a Starling valve which controlled the inlet of air so that the pressure in the tank was kept at approximately —500 mm. of mercury. The animals were taken out of the tank only once daily for about an hour to allow for feeding and

TABLE 2
Effect of length of time after operation upon change in weight of heart and lung

NUMBER RATS	AVERAGE BODY WEIGHT	NORMAL TOTAL LUNG WEIGHT	TOTAL LUNG WEIGHT DAYS AFTER OPERATION					HEART WEIGHT AFTER OPERATION									
			At 3 days		At 7 days		At 14 days		At 30 days		Per cent normal	Normal	At 7 days	At 14 days	At 30 days	Per cent normal	
	<i>gm.</i>	<i>mg.</i>															
6	118	660	405														
6	199	1080	563														
7	78	540		552													104
7	199	1080		768													105
20	105	600			605												101
20	207	1100			1110								410	685			102
7	127	700														547	119
6	164	800														727	123
6	226	1180															
4	326	1640														955	103

watering. The changes in pressure were made very slowly. After a few days the rate of change could be speeded up. The animals remained in the tank for 14 days. The results shown in table 3 demonstrate that the lungs of animals kept at low atmospheric pressures are heavier than the normals, and that the lobectomized rats have lung weights equal to the controls.

The effect of decreasing the size of the thoracic cage. In order to decrease the pull on the remaining lung after operation, a bolus of wax was inserted intrapleurally at the time of lobectomy. At the end of the 14-day period it was seen that the remaining lung tissue fell below the predicted total lung weight. The amount of deviation corresponded roughly to the size of the intrathoracic mass. In contrast to the close

TABLE 3

The effect of low atmospheric pressure on lobectomized and unoperated rats

Number rats	Average body weight gm.	Observed heart weight mg.	Per cent change over normals	Observed total lung weight mg.	Per cent change over normal at atmospheric pressure
4 (unoperated)	180	814	+27	1688	+41
4 (operated)	84	464	+24	854	+40
4 (operated)	135	551	+18	1164	+40

correlation of the increase of the total lung weight in each individual rat in the low atmospheric pressure experiment, there was a wide range of variation in the rats in which wax had been placed in the thorax. This difference was considered to be due to the constancy of the stimulus in the former case as opposed to the irregularity of the amount of change in thoracic volume in the wax experiment, because of the irregularity of the size of the wax bolus.

There was always a certain amount of infection around the wax although it seemed fairly well encysted. Attempts to repeat the experiment using oil also were unsatisfactory because of the inflammatory reaction, thus making the significance of weight changes questionable.

Thoracoplasty. In order to exclude the effect of infection, further attempts were made to lessen the stretch on the lungs without the use of a foreign substance. The first attempt was by means of thoracoplasty. However, it was technically impossible to remove more than five ribs at one sitting because of the thinness of the pleura; for many small holes were torn in the pleura during the course of the procedure, thereby causing enough of a pneumothorax to endanger the life of the animal. Yet even though the decrease in the thoracic volume was small, the total lung weights at the end of the 2-week

TABLE 4
Effect of decreasing intrathoracic volume by wax

Number rats	Average body weight gm.	Observed total lung weight mg.	Average weight of wax mass gm.	Per cent deviation below normals
4	90	510	6.87	-13
5	122	561	5.56	-21
6	178	804	6.21	-18

TABLE 5
Effect of decreasing intrathoracic volume by thoracoplasty

Number rats	Average body weight gm.	Average total lung weight mg.	Predicted total lung weight mg.	Per cent deviation
9	212	1036	1100	-6
8	177	924	960	-4

period are seen to fall below the normal level for rats of similar body weight. The significance of such a small deviation in lung weight might be disregarded if the results were not consistent with the other experiments in which thoracic volume was reduced.

Phrenic avulsion. The left phrenic nerve was avulsed just above the diaphragm through a low left intercostal incision. The left phrenic nerve was chosen because it is free of vessels whereas the right phrenic is closely associated with the vena cava. The rat will not survive avulsion of both phrenics.

Before the animals were killed at the end of the 14-day period, the success of the procedure was checked by fluoroscopy, by absence of motion of the left diaphragm when the abdomen was opened, and finally by dissecting out the course of the nerve. The data presented in table 6 show that avulsion of the left phrenic nerve caused a fairly constant lag in the increase of lung weight as compared to the growth of the lungs of normals of the same body weight.

Pneumoperitoneum. In order to enforce the effect of phrenic avulsion, rats were given intraperitoneal injections

TABLE 6

Effect of decreasing the intrathoracic volume by phrenic avulsion

<i>Number rats</i>	<i>Average body weight gm.</i>	<i>Average total lung weight mg.</i>	<i>Predicted total lung weight mg.</i>	<i>Per cent deviation</i>
20	110	521	610	—15
17	166	797	900	—11
17	190	810	1000	—19

TABLE 7

*Effect of decreasing the intrathoracic volume by phrenic avulsion and
pneumoperitoneum*

<i>Number rats</i>	<i>Average body weight gm.</i>	<i>Average total lung weight mg.</i>	<i>Predicted total lung weight mg.</i>	<i>Per cent deviation</i>
12	225	995	1160	—14

of air for the 2-week period following phrenic avulsion. Small doses were given at first, gradually being increased so that a rat as large as 200 gm. was receiving 100 cc. every other day. More air than this seemed to prevent the animals from feeding. In the series in which the combination of these procedures was tried, there was no further deviation in the lung weight than in the series having phrenic avulsion alone. When, however, either or both procedures were combined with removal of lung tissue (table 8), there was a further small deviation in the observed lung weight as compared to the normals after the 14-day postoperative period.

In another group of rats treated in a similar fashion to those in table 8, the air was aspirated at the end of the 14-day period, and the rats allowed to live 7 days longer, a period of 21 days following operation. The lung weights in these animals more closely approached the normal (table 9).

TABLE 8
Effect of decreasing intrathoracic volume by combination of procedures

<i>Number rats</i>	<i>Procedure</i>	<i>Average body weight gm.</i>	<i>Predicted total lung weight mg.</i>	<i>Observed total lung weight</i>	<i>Per cent deviation</i>
12	Lob, pn, phr	202	1060	822	—22
13	Lobec, pn	208	1090	867	—20

TABLE 9
Effect of decreasing then increasing the intrathoracic volume

<i>Number rats</i>	<i>Average body weight gm.</i>	<i>Average total lung weight ma.</i>	<i>Predicted total lung weight mg.</i>	<i>Per cent deviation</i>
15	133	685	740	—8
5	208	990	1080	—8

DISCUSSION

The following hypothesis would fit the data presented. The rat has more than three times the amount of lung tissue necessary for life during its growing stage. Even with the removal of more than two-thirds of the lung tissue, there still remains adequate tissue to aerate the blood satisfactorily. However, the shift of the mediastinum and the pull of the diaphragm and chest wall, as well as the negative pressure exerted on the remaining lung as the air is absorbed from the operated side, causes the remaining lung to be stretched. The lung responds by a growth of more tissue. Growth stops when the normal intrapleural pressure is reached. If these forces are partially or completely prevented from acting upon the remaining lung, the increase in weight lags accordingly. If, on the other hand, these forces are accentuated, the increase in weight surpasses the normal. The lung will fit the thoracic cage and, in the growing animal, it does so by actually adding tissue.

Whether the increase in weight represents the formation of new alveoli or simply an increase in size of those alveoli already present is being investigated at the present time.

That the lung weights are actually a measure of the protein content was shown by Addis ('28) who demonstrated that the weight was parallel to the nitrogen content of the lung. The error due to the increased amount of blood was partially corrected by bleeding the animals and washing with salt solution.

CONCLUSIONS

1. In the growing rat, the lung weight is proportional to the body weight.

2. Removal of lung tissue in small or large amounts is followed by restitution of lung weight proportional to the body weight of the animal, so that at the 14-day interval, the lung weight is equal to the normal weight for rats of the same body weight.

3. Stretching the lung increases its weight; interfering with the stretch on the lung reduces the amount of increase in weight of the lung of the growing rat, and also reduces the rate of increase after lobectomy.

4. The younger the animal, the more rapid is the rate of restitution of lung weight.

5. The increase in lung weight is a response to a purely mechanical stimulus.

It is a pleasure to thank Dr. William Dock and Dr. Thomas Addis for many suggestions and advice. Mr. William Lew was of indispensable technical assistance.

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THE REDUCTION OF OSMIC ACID AS AN INDICATOR OF ADRENAL CORTICAL ACTIVITY IN THE RAT

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TWO PLATES (SIXTEEN FIGURES)

INTRODUCTION

Despite several attempts to correlate one or another cytological change in the cells of the adrenal cortex with its state of activity, there is no well-defined method whereby the activity of the gland can be judged by its histological appearance. The practical importance of such a procedure is obvious. The observations presented below are concerned with the variation of those elements of the cortical cells which reduce osmic acid under conditions known to depress (administration of cortical hormone) or to stimulate (cold, heat, drugs) the activity of the gland. These observations have made it possible to define cytological changes characteristic of different degrees of activity of the adrenal cortex of the rat.

MATERIAL AND METHODS

Except for a series of guinea pigs which were injected with thyrotropic hormone, the white rat was used as the experimental animal. Litter mates matched for weight and sex were used for each series of experiments. The animals were sacrificed by decapitation or by a sharp blow on the head. Unless otherwise mentioned, the adrenals were then removed, cut in two, and immediately placed in fixative.

Maximow's osmic acid fixative as modified by Gersh ('39) was used in the dark. The small pieces of adrenal were placed in about 5 cc. of a solution consisting of 1 part of 25% neutral formalin, 9 parts of Zenker's fluid without acetic acid, and 1.5 parts of 2% osmic acid. This solution was renewed after 24 hours. At the end of the next 24 hours the tissue was rinsed well in six changes of distilled water. It was then transferred to 2% osmic acid for 24 hours, washed in twelve changes of distilled water over a period of $\frac{1}{2}$ hour, and dehydrated by immersion in 80%, 95% and absolute alcohol successively for $\frac{1}{2}$ hour. From the absolute alcohol it was transferred to a mixture of equal parts of absolute alcohol and xylol for 15 minutes; then to xylol for 15 minutes and finally embedded in 62°C. paraffin. Sections were cut at 3 or 9 μ . They were passed through iodine-alcohol. Some blocks were fixed in Zenker's formol and stained with hematoxylin and eosin.

In all the experiments, except where noted, animals had free access to the standard McCollum diet and water. They were maintained at a uniform room temperature of $80 \pm 5^\circ$.

RESULTS

The reduction of osmic acid by the normal adrenal gland. On the basis of their reduction of osmic acid five zones (plate 2, fig. 1) can be distinguished in the adrenal cortex of the normal white rat. The most peripheral of these consists of the glomerulosa plus a thin layer of cells, varying slightly in its thickness, of the underlying fasciculata. This zone will be referred to as the peripheral zone. Immediately beneath this peripheral zone and lying in the fasciculata, in the normal animals of the ages studied here, is a zone almost free of substances reducing osmic acid; this zone will be designated the clear zone. The remaining fascicular zone will be divided into two portions: approximately the outer two-thirds contains considerable reducing substance and will be referred to as the outer fascicular zone; the inner portion of the fasciculata, normally poor in reducing substances, will be desig-

nated as the inner fascicular zone. Finally, there is the thin reticular zone lying next to the medulla.

Adrenal cortical hormone series. The most certain method for reducing the activity of an endocrine gland is to administer the hormone elaborated by that gland. To avoid the incidental effects, inevitable when one injects a crude extract into animals, we gave the hormone by mouth, exclusively. Animals 1 month of age were used in order to minimize the quantities of hormone necessary for the experiment. The adrenal cortical hormone was assayed on adrenalectomized rats (Grollman, '36) and incorporated with a weighed amount of the animal's food. Either a charcoal adsorbate, or a purified concentrate prepared by the methods described elsewhere (Grollman, '36, '39) or commercially available forms of these products were utilized.¹

The results of the administration of the cortical hormone are given in table 1 and figures 2, 3 and 4 of plate 1. In a dosage of 0.5 unit² a day for 3 days the hormone produced the first definitive change from normal. This change consisted of a diminished reduction of osmic acid in the peripheral and clear zones. A dosage of 2 units a day (plate 1, fig. 3) for 3 days practically abolished reduction in the peripheral and clear zones and in addition decreased the reducing substances in the outer fascicular zone. Six units given daily for 4 days (plate 1, fig. 4) almost completely eliminated reducing substance from all zones except the outer fascicular where, however, it was definitely diminished.

It appeared that the zones in order of sensitiveness to the cortical hormone, as measured by changes in the osmic acid reducing substances, were first the peripheral and clear zones; second, the fascicular and reticular zones. Complete dis-

¹ We are indebted to Dr. Ernest Blanchard of Schiefflin and Company, and to Dr. George Cortland of the Upjohn Company, for a supply of part of the hormone used in the present investigation.

² The term unit as used here is that amount which suffices to maintain normal growth in an adrenalectomized month old rat. This amount of hormone is equivalent, when tested in this manner, to 1 mg. of desoxycorticosterone acetate or 0.01 mg. of the most potent crystalline product obtainable (Grollman, '39).

TABLE 1

The variation in the reduction of osmic acid by the various zones of the adrenal cortex of the white rat under several experimental conditions. P.Z., C.Z., F.Z., and R.Z., refer respectively to peripheral zone, clear zone, fascicular zone and reticular zone.

0, indicates no reduction of osmic acid; tr, a trace of reduction, +, 2+, 3+ and 4+, increasing degrees of reduction, respectively

TREATMENT	P.Z.	C.Z.	OUTER F.Z.	INNER F.Z.	R.Z.
1-month-old control	2+	+	2+ to 3+	0 to +	0 to +
Cortin, 0.25 unit for 3 days	+	tr	2+	0 to tr	0 to +
Cortin, 0.5 unit for 3 days	0 to +	tr	2+ to 3+	0 to +	0 to +
Cortin, 2 units for 3 days	0	0	+	0 to tr	0 to +
Cortin, 6 units for 4 days	0	0	+	0	0
4-month-old control	2+	tr	2+	tr to +	tr to +
Cold (10°C.) for 0.5 hour	2+ to 4+	+	2+ to 3+	2+	+ to 2+
Cold for 1 hour	3+ to 4+	2+	3+	+	+
Cold for 2 hours	4+	3+	3+ to 4+	tr to 3+	+ to 2+
Cold for 4 hours	4+	2+ to 3+	3+ to 4+	3+	2+
Cold for 22 hours	0 to 2+	tr	tr to 2+	tr	0
4-month-old control	2+	tr	2+	tr to +	tr to +
Heat (39°C.) for 0.5 hour	4+	3+	3+	+ to 2+	2+
Heat for 1 hour	4+	4+	4+	+ to 2+	3+
Heat for 3 hours	3+	tr to +	+	+	+
Heat (39°) for 6 hours and (37°) for 4 hours	0 to 2+	0 to +	0 to tr	0	0
4-month-old control	2+	tr	2+	tr to +	tr to +
Hypertrophy, 1 day	2+ to 4+	2+ to 4+	2+ to 3+	tr to +	tr to +
Hypertrophy, 4 days	3+ to 4+	3+ to 4+	2+ to 3+	2+	2+
Hypertrophy, 8 days	4+	4+	4+	+ to 3+	3+
Hypertrophy, 13 days	4+	4+	4+	3+ to 4+	3+ to 4+

appearance of reducing substances occurred last in the outer fasciculata.

Cold series. Litter mates, 4 months old, were placed in a room at 10°C. for periods up to 22 hours. The effect of this environment on the content of substances reducing osmic acid is shown in table 1 and in the photomicrographs of plate 2 (figs. 2 to 4). Cold of this degree produced an effect obvious at the end of $\frac{1}{2}$ hour. The most constant change consisted of an increase in reduction by the peripheral and clear zones; there was also, in some instances, a definite increase in the inner fascicular and reticular zones. Exposure to cold for 1 hour further increased the reducing substances in the peripheral, clear and outer fascicular zones without producing constant changes in the other zones. Additional cold, up to a period of at least 4 hours (plate 2, fig. 3) produced its most pronounced change by an increase in reduction by the fascicular and the reticular zones. Animals in the cold for periods approaching 1 day (fig. 4) showed a very marked loss of reducing substance. This was evident in all zones; but the largest quantity of reducing substance was retained by the peripheral zone.

It appeared that the zones in order of sensitiveness to cold as measured by changes in substances reducing osmic acid were the peripheral and clear, the outer fascicular, and finally the inner fascicular and reticular zones.

Heat series. Litter mates, 4 months old, were exposed to temperatures from 37 to 40°C. for various periods up to 10 hours. This elevation of temperature produced a marked effect at the end of $\frac{1}{2}$ hour (table 1 and plate 2, fig. 5). Substances reducing osmic acid increased in all zones with the most marked effect evident in the peripheral and clear zones. Exposure to heat for 1 hour produced no further noteworthy difference beyond an intensification of reduction in the outer fasciculata. A marked change was, however, noted in animals which were kept at these high temperatures for from 3 to 5 hours (fig. 6). This change consisted of almost complete loss of reducing substance from all portions of the

cortex except the peripheral zone and the inner portion of the outer fasciculata. The adrenals (figs. 7 and 8) from a prostrate animal kept at high temperature for 10 hours showed either no reducing substance or reducing substance limited to the peripheral zone.

The different zones reacted to heat in essentially the same order as to cold excepting that, at the temperatures used, the reaction to cold took relatively longer to manifest itself.

Hypertrophy series. A series of 4-month-old animals was unilaterally adrenalectomized and the remaining adrenal removed at various periods after the adrenalectomy. The results are shown in table 1 and plate 1 (figs. 5 to 7). The earliest change noted, 1 day following adrenalectomy, was an increase in reducing substance in the peripheral and clear zones of the remaining adrenal. This had become striking by 8 days (fig. 6). Increase of reducing substance in these zones was followed by definite increase in the outer fascicular, reticular and inner fascicular zones in the order named. All zones showed an almost uniformly great increase of reducing substance within 2 weeks (fig. 7). The different zones reacted to hypertrophy in the same order as to heat and cold.

Other observations. Variations, usually slight but at times marked, were observed between the two adrenal cortices of an experimental animal and in the reaction of different parts of the same cortex in response to cortin, cold, heat and hypertrophy. When the two glands showed differences in reaction, at times the right, and at other times the left gland was the more reactive. The cortices of several normal animals older than a year were examined; they showed considerable variation in reducing substance; in most instances there was less reducing substance in the peripheral and outer fascicular zones than in the 4-month-old animals. The clear zone in animals 1 month old contained somewhat more reducing substance than that of animals 4 months old (fig. 1, plates 1 and 2).

Substances reducing osmic acid were also studied in several other circumstances without any attempt to produce other

than a marked effect. Thyrotropic hormone³ produced a general increase in the reducing substance of the cortex of the guinea pig adrenal; injection of tetrahydro- β -naphthylamine and inanition (plate 1, fig. 8) due to starvation gave large increases in the reducing substances in the cortex of the rat.

The histological picture of the adrenal was not affected if the animal, after death and before fixation, was kept at room temperature for 2 hours and then for 20 hours in the ice box.

DISCUSSION

The experimental conditions described above have been chosen with the view of reducing or increasing adrenal cortical activity from the normal. The adrenal cortex of an animal given cortical hormone will be inhibited, for in the presence of an extraneous supply of its hormone, the cortex will deliver less hormone to the animal. The other procedures utilized in the above-described experiments all stimulate adrenal cortical activity. In the case of unilateral adrenalectomy, the second gland hypertrophies and maintains a normal supply of the vital hormone. Extremes of temperature also stimulate the activity of the adrenal cortex. This has been demonstrated by 1) the hypertrophy of the glands which follows subjecting an animal to these conditions, 2) the additional hormone required to maintain animals following adrenalectomy as compared to that necessary when such animals are maintained at the normal room temperature and 3) the short survival period of adrenalectomized animals maintained at abnormal temperatures (Grollman, '36). Inanition, also, as judged by the same criteria stimulates adrenal cortical activity although conditions here are probably complicated by the incidental metabolic disturbances. The injection of tetrahydro- β -naphthylamine, which is a sympathetic stimulant, or of the thyrotropic hormone of the anterior pituitary

³ We are indebted to Ayerst, McKenna and Harrison for some of the thyrotropic hormone used. The remainder was prepared in the laboratory by alkaline extraction of the anterior pituitary gland of cattle.

gland, also cause hypertrophy of the adrenal and increase cortical activity secondary, probably, to the increased metabolism which these substances induce (Grollman, '36).

Stimulation of cortical activity causes an increase of osmic acid-reducing substances; reduction of activity, a decrease in these substances. Ingle, Higgins and Kendall ('38), who gave rats massive but unassayed doses of cortical extract, also noted the complete disappearance of cortical lipoid under these conditions. The change in concentration of reducing substance, moreover, is rather accurately proportional to the extent of exposure to the experimental condition so that the histological picture gives a measure of the degree of reduction or increase of activity.

The different zones of the cortex of the white rat vary in their response to an altered environment. The most reactive zones are the peripheral and clear; they show a large increase of reducing substances with adequate stimulation and a complete loss of reducing substance with adequate inhibition. The next most reactive zone is the outer fascicular and it is followed by the less reactive reticular and inner fascicular zones which respond about alike. Though their response is delayed, the reticular and inner fascicular zones can, with adequate stimulus, show a magnitude of reaction almost as great as the other zones. This marked response of the reticularis makes doubtful the validity of the generally accepted view that it is an area of the cortex made up of dying cells (Grollman, '36).

Prolonged periods of exposure to heat or cold produce a decrease of substances reducing osmic acid which is interpreted as indicative of exhaustion. In the one prostrate animal exposed to high temperature for 10 hours, for example, one adrenal was practically without reducing substance and the other showed reduction only in the peripheral zone.

It is clear, consequently, that stimulation or inhibition of cortical activity produces characteristic changes not to be confused with one another except in extreme cases. These extreme cases involve complete loss of reducing substances due

to exhaustion of the stimulated cortex or complete loss of reducing substances due to suppression of activity by large doses of cortical hormone. Confusion may, however, arise from changes due to metabolic disturbances which may give a histological picture like that found in a highly stimulated gland.

The substances responsible for the reduction of osmic acid in the adrenal cell are of two varieties. The unsaturated constituents of the visible droplets of lipoid will reduce osmic acid and deposit the lower oxides of osmium on the surface or within the interior of these droplets. Besides these visible droplets other reducing substances are present in the cytoplasm of the cell which also cause the deposition of reduced osmic acid (Hoerr, '36). These reducing substances comprise such compounds as ascorbic acid and glutathione, which are present in large quantities in the adrenal, the cortical hormone, and probably other, as yet unidentified, reducing substances.

It should be emphasized that the definite correlation which we have shown to exist in the rat between the reduction of osmic acid and cortical activity cannot yet be transferred to other species. For example, in the mouse, experiments (Gersh and Grollman, '39) undertaken from a point of view different from that presented here, may at first glance present a contradiction to our findings on the rat. In the mouse, small doses of cortical hormone produce apparently inconstant changes in the cortical reducing substances. It is well known (Whitehead, '33), however, that the lipoid content of the adrenal in the mouse is highly variable. Correlation of concentration of reducing substances with state of activity, consequently, must in the case of the mouse involve a large series to be treated by statistical methods or a smaller series composed of highly uniform individuals. Moreover, since lipoid and other reducing substances are not conspicuous cytological inclusions in the adrenal cortex of all species (Elliott, '14), it is realized that the results obtained in the rat are probably not applicable to all other mammals. Undoubtedly, the degree of uni-

formity of our own experiments is attributable in part to the maintenance of constant experimental conditions and the use of a highly standardized experimental animal.

SUMMARY

The cytological changes occurring in the adrenal cortex of the rat as revealed by the reduction of osmic acid have been correlated with changes in the activity of the gland. Reduction in adrenal activity was induced by the administration of various amounts of the cortical hormone; activity was stimulated by unilateral adrenalectomy, heat, cold, the injection of tetrahydro- β -naphthylamine, thyrotropic hormone, or inanition. The changes in reduction of osmic acid by the cortical cells occurring under these various conditions are described as they occur in the various zones of the adrenal. In general, depression of activity is correlated with a decrease in the reduction of osmic acid; stimulation of activity, with an increase in the reduction of osmic acid.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of sections of the adrenal gland to show the effects of the continuous administration by mouth of the adrenal cortical hormone, of hypertrophy of the remaining gland following unilateral adrenalectomy, and of inanition. The preparations were fixed in osmic acid, sectioned at 9μ and photographed unstained. $\times 60$.

- 1 Untreated control. Age 4 weeks. The peripheral, clear, outer fascicular, inner fascicular and reticular zones are indicated respectively by a, b, c, d and e. The medulla is indicated by m.
- 2 Adrenal cortical hormone ($\frac{1}{2}$ unit) administered for 3 days. Age 4 weeks.
- 3 Adrenal cortical hormone (2 units) administered for 3 days. Age 4 weeks.
- 4 Adrenal cortical hormone (6 units) administered for 4 days. Age 4 weeks.
- 5 Effect of unilateral adrenalectomy on the remaining adrenal, 4 days subsequently. Age 4 months.
- 6 As in figure 5 after 8 days.
- 7 As in figure 5 after 13 days.
- 8 Effect of 2 days inanition on a 4-month-old rat.

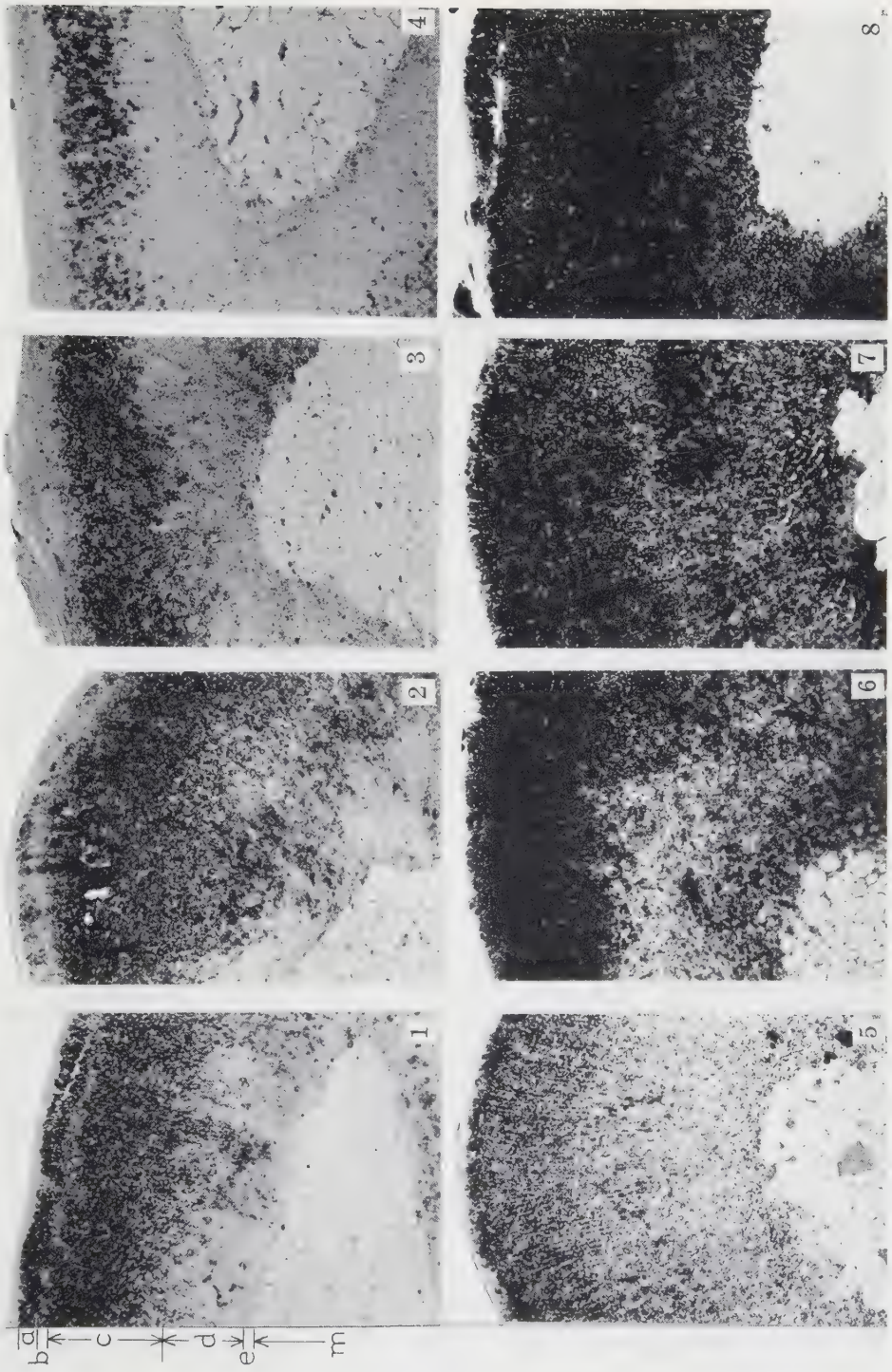
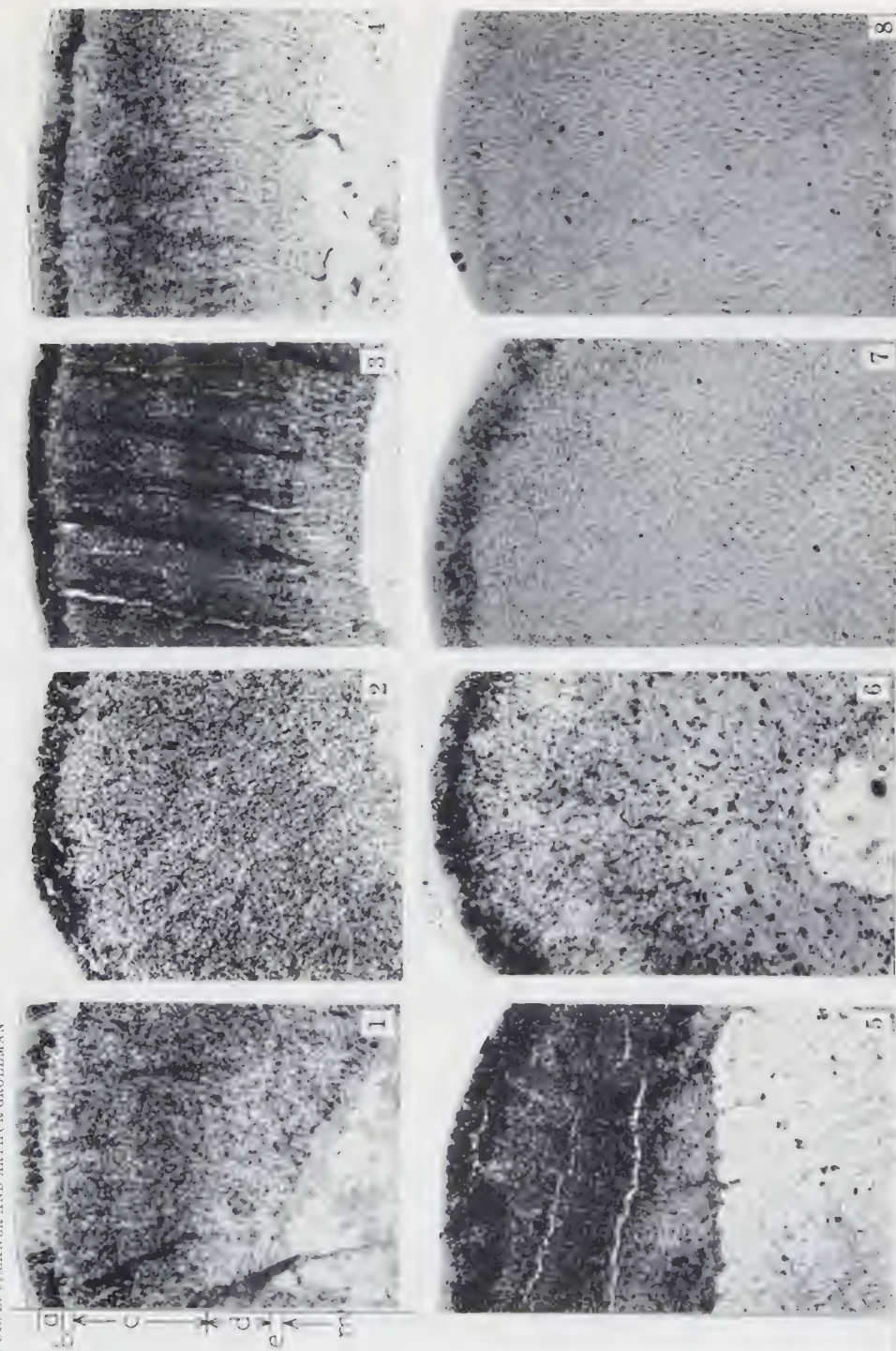


PLATE 2

EXPLANATION OF FIGURES

- 1 Normal 4-month old control. a, b, c, d, e, and m have the same indications as in figure 1, plate 1.
- 2 Effect of cold ($10^{\circ}\text{C}.$) for 0.5 hour. Age 4 months.
- 3 Same as figure 2 but for 4 hours.
- 4 Same as figure 2 but for 22 hours.
- 5 Effect of heat ($37^{\circ}\text{C}.$) for 0.5 hour. Age 4 months.
- 6 Same as figure 5 but for 3 hours.
- 7 Same as figure 5 but for 10 hours. (Right adrenal.)
- 8 Same as figure 5 but for 10 hours. (Left adrenal.)



MATURATION AND CLEAVAGE FIGURES IN OVARIAN OVA¹

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THREE PLATES (FOURTEEN FIGURES)

While studying a series of guinea pig ovaries in which ovulation had been induced by injections of luteinizing hormone preparations, it was noticed that all medium and large follicles were undergoing atresia or luteinization, and that almost without exception, maturation spindles were present in the ova. Closer examination revealed that in many the maturation spindles were abnormal. Tripolar and multipolar mitoses, as well as fragmentation of the egg or of the nucleus, were observed.

These findings prompted a re-investigation of the ovaries from normal animals at various stages of the cycle (Myers, Young and Dempsey, '36). The results are thought to throw light on the occurrence of maturation figures and the fate of atretic ova, and are presented in the following sections.

MATERIAL AND METHODS

A large series of guinea pig ovaries, removed at known stages in the reproductive cycle, was available for this study. The condition of these ovaries has been related to the oestrous behavior and to the reproductive cycle of the respective animals in previous reports (Myers, Young and Dempsey, '36;

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Young, Dempsey, Myers and Hagquist, '38). The ovaries of animals in which various experimental procedures had been carried out were also studied. These procedures included intravenous injections of luteinizing hormone, daily injections of oestrin and of progesterone, hypophysectomy, removal of corpora lutea, and other experimental disturbances (Dempsey, Hertz and Young, '36; Dempsey, '37).

The ovaries were fixed in Bouin's fluid or in Zenker-formol; dehydration was effected and serial sections 10 μ in thickness were prepared. These sections were then stained in either iron hematoxylin and orange G or in Delafield's hematoxylin and eosin.

RESULTS

Maturation figures. In the ovaries from ten guinea pigs which had been injected with luteinizing hormone preparations (sheep luteinizing hormone, pregnant mare serum, pregnancy-urine extract) on the twelfth to fourteenth days of the cycle, ovulation points and corpora lutea were macroscopically visible at autopsy 1 to 4 days after the injections. Histological examination of these ovaries confirmed the presence of corpora lutea and showed, in addition, that all medium and large follicles remaining in the ovaries were undergoing lutein changes. The ova in these luteinizing follicles almost invariably contained maturation figures. In some cases polar bodies could be found, while in others the figures were apparently first maturation spindles, as no polar bodies could be seen.

Many of the maturation figures seen in these follicles were abnormal. Splitting of the spindle into two or three parts, arranged in a V- or W-shaped fashion, is the most common type of aberration (figs. 3 and 5). Loss of chromatin material from the equatorial plate by detachment from the spindle fibers is also quite common. These dislocated chromatin bodies are sometimes found floating free in the cytoplasm, or may be enclosed in a vesicle which separates them from the chromatin of the equatorial plate and from the surrounding cytoplasm. An aberration of this type is illustrated in figure 2.

Multipolar mitotic spindles also may be found. They are less common than the above types of maturation abnormalities, but are encountered with considerable regularity. The chromatin material in these maturation figures may be rather equally divided between several spindles (figs. 4 and 7), or may be arranged, for the most part, on a single spindle with only a few chromatin bodies aligned on smaller, secondary spindles (fig. 6). These figures may be completely separate and distinct (fig. 4), or they may be distributed among three or more common condensation centers (figs. 6 and 7).

Similar maturation figures may be observed in the ovaries of normal animals. Beginning at about the time of ovulation in the guinea pig, atresia and degeneration of all medium and large follicles occur and a new crop of follicles begins its development. During this period of atresia, maturation figures may be seen in the ova. These figures may appear quite normal soon after ovulation, but later may be as aberrant as those illustrated after injection of luteinizing hormone preparations. Figure 4 shows a double maturation figure from a normal animal killed 2 days after ovulation.

Maturation figures, although most abundant after injections of L.H. or after ovulation, may also be found in ovaries after a variety of experimental procedures. In general, it may be said that maturation figures are present in ova from atretic follicles, regardless of the experimental procedure employed. Thus, maturation figures have been observed in animals injected daily with oestrin, in animals injected daily with progesterone, and even in the ovaries from hypophysectomized animals (fig. 9).

Cleavage divisions. In all of the animals studied, maturation figures, usually in metaphase stages but also observed in prophase and telophase, occur much more frequently than do cleavage divisions of the ovum. Dividing cells are encountered, however, as illustrated in figures 11, 12 and 13. A complete series from the single-celled to multi-celled, morula-like structures can be seen. Many of these structures are no longer viable and are undergoing resorption, as is shown in

figure 12. However, the presence of a continuous series of figures in gradually increasing complexity indicates that division of these atretic ova into many-celled egg masses does occur.

Figure 14 illustrates a still later stage which may be encountered. This structure consists of an inner layer of cuboidal cells and an outer layer of giant cells surrounded by what appears to be theca interna. No granulosa cells or lutein cells are distinguishable. Hemorrhage into the central cavity has occurred, apparently due to erosion of the blood vessels surrounding the giant cell layer.

This structure bears a striking resemblance to certain fetal components of the placenta. In particular, the giant cell layer appears strictly comparable to the trophoblastic giant cells which are found in the embryonic placentae of rodents. Such cells have been described in guinea pig ovaries by Leo Loeb ('32) and have been designated by him as 'chorionic wander cells.' Likewise, the whole structure is quite comparable with the figures illustrated by Loeb, which he called embryonal placentomata.

There is only slight evidence of a decidual reaction in the ovarian stroma surrounding the giant cell layer. Some erosion and cytolysis of the theca interna have occurred at the points of contact between it and the giant cell layer. Likewise, erosion of the blood vessels by the giant cells may be observed, thus accounting for the hemorrhage into the central cavity of the figure.

DISCUSSION

A large number of ovarian ova contain maturation spindle figures after the action of luteinizing hormone at ovulation and after the injection of luteinizing hormone at other times in the cycle. It is doubtful, however, if luteinizing hormone is released from the pituitary at the tenth to fourteenth days of the reproductive cycle; it has been shown experimentally that progesterone inhibits the release of luteinizing hormone, and hypophysectomy removes the source of pituitary hor-

mones. Nevertheless, since maturation figures have been observed in ova after these procedures, luteinizing hormone cannot be the sole factor which initiates maturation processes.

Friedgood and Pincus ('35) and Pincus ('36) concluded that the presence of maturation figures in the ova of pre-ovulatory rabbit follicles is indicative of sub-ovulatory amounts of pituitary hormone. The atresia and maturation changes which occur during the follicular growth phase, on the contrary, can best be explained as a lack of pituitary stimulation, according to Pincus ('36). The occurrence of maturation figures in the ovaries of animals, in which the presence or absence of the pituitary gonadotropic hormones has been controlled experimentally, indicates that, in the guinea pig at least, maturation changes may occur in atretic follicles quite irrespective of whether or not the pituitary gland is functionally active. Consequently, although marked atresia and maturation occur as a result of luteinizing hormone action, the presence of maturation figures is of doubtful value as a criterion of the presence of luteinizing hormone.

The presence of atypical mitotic figures is a generally accepted cytological sign of degeneration. Nevertheless, the abnormal mitoses observed in the guinea pig ovaries are not severe enough to prevent cleavage and subsequent differentiation into multicellular figures (figs. 11, 12 and 13). Likewise, Sneider ('37) has described a late telophase stage in which a tripolar mitotic figure divided into three daughter cells.

Division of the ovarian ova may or may not be regular and equal; usually it is atypical and results in multinucleate cells or unequal blastomeres. These cleaving blastomeres, like the atretic ova, undergo ultimate degeneration and resorption. Nevertheless, in occasional eggs which escape degenerative changes for relatively long periods, the cleavage divisions may lead to the formation of quite complex structures (figs. 13 and 14). Regardless of whether this mode of cleavage can be considered to be true parthenogenesis, the structures formed simulate to a remarkable degree the early developmental stages of fertilized eggs.

SUMMARY AND CONCLUSIONS

Normal and abnormal maturation figures are observed in large numbers in the ova of atretic follicles from guinea pigs killed shortly after ovulation or after injections of luteinizing hormone. Similar maturation figures may be seen, but less frequently, in atretic ova from guinea pigs killed at other times in the reproductive cycle, after daily injections of progesterone and after hypophysectomy. Since progesterone inhibits the release of luteinizing hormone and hypophysectomy abolishes all pituitary gonadotropic hormones, it is concluded that the pituitary hormones are not essential for the initiation of maturation changes in ova.

These ovarian ova may divide and differentiate into quite complex structures. Occasional structures containing giant cells, morphologically similar to trophoblastic giant cells, occur. These structures ultimately undergo degeneration and are re-absorbed.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Maturation figure in an ovum from a guinea pig, 26 hours after injection of pregnancy urine extract. The spindle figure has moved somewhat toward the center of the ovum; otherwise the figure is essentially normal. Iron hematoxylin and orange G. $\times 1100$.

2 Maturation figure, 42 hours after injection of 0.2 gm. equivalent of sheep luteinizing hormone. Two chromatin bodies are separated from the metaphase plate. The vesicle at the top of the figure containing chromatin material probably represents an abnormal first polar body. Iron hematoxylin and orange G. $\times 1100$.

3 Maturation figure from the same animal described in figure 2. The chromatin material is distributed unevenly on the metaphase plate, and fibers and chromatin material have split away from the spindle figure. Iron hematoxylin and orange G. $\times 1100$.

4 Double maturation figures in an ovum from a normal, unmated animal killed 2 days after oestrus. Both spindles are somewhat smaller than usual; otherwise they show no obvious abnormality. Hematoxylin and eosin. $\times 1100$.



PLATE 2

EXPLANATION OF FIGURES

5 W-shaped, split maturation figure from an animal killed 42 hours after the injection of 0.2 gm. equivalent of sheep luteinizing hormone. Iron hematoxylin and orange G. $\times 1100$.

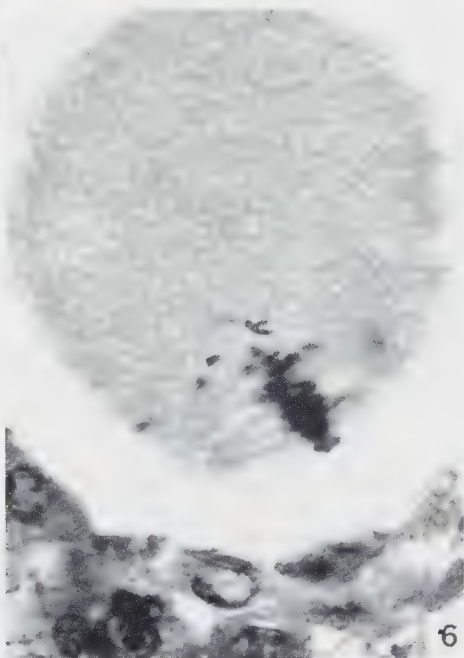
6 Tripolar maturation figure in an animal killed 42 hours after the injection of 0.2 gm. equivalent of sheep luteinizing hormone. The majority of the fibers and chromatin material are distributed on one of the three spindles, while the other two are composed of only a few spindle fibers and chromatin bodies. Iron hematoxylin and orange G. $\times 1100$.

7 Multipolar maturation figure from an animal killed 26 hours after the injection of 50 R.U. pregnancy urine extract. The chromatin material and spindle fibers are distributed more or less equally among the five spindles which are present in this figure. Hematoxylin and eosin. $\times 1100$.

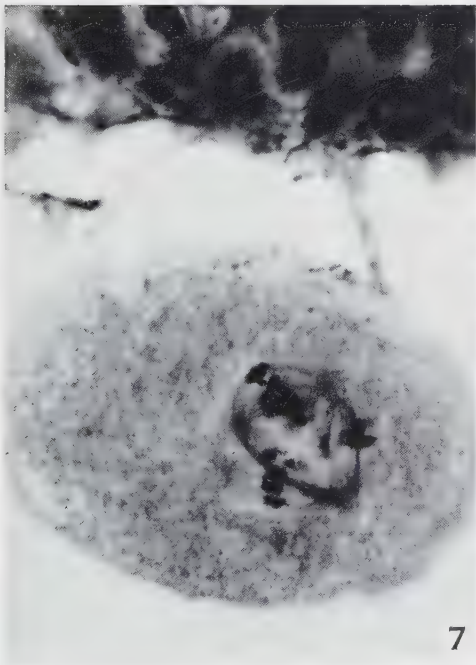
8 Disintegrating maturation figure from an animal killed 49 hours after the injection of 0.2 gm. equivalent of sheep luteinizing hormone. Iron hematoxylin and orange G. $\times 1100$.



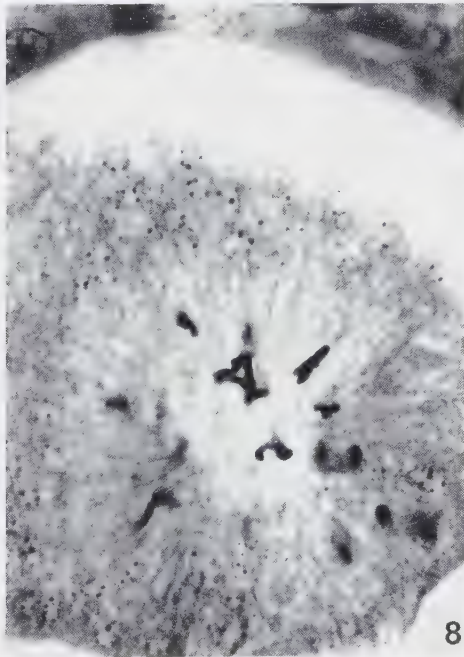
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PLATE 3

EXPLANATION OF FIGURES

9 Maturation spindle from ovary of an animal killed 12 days after hypophysectomy. Cytoplasmic strands connecting ovum and follicular cells, and a mitotic figure in the granulosa may be seen. Hematoxylin and eosin. $\times 725$.

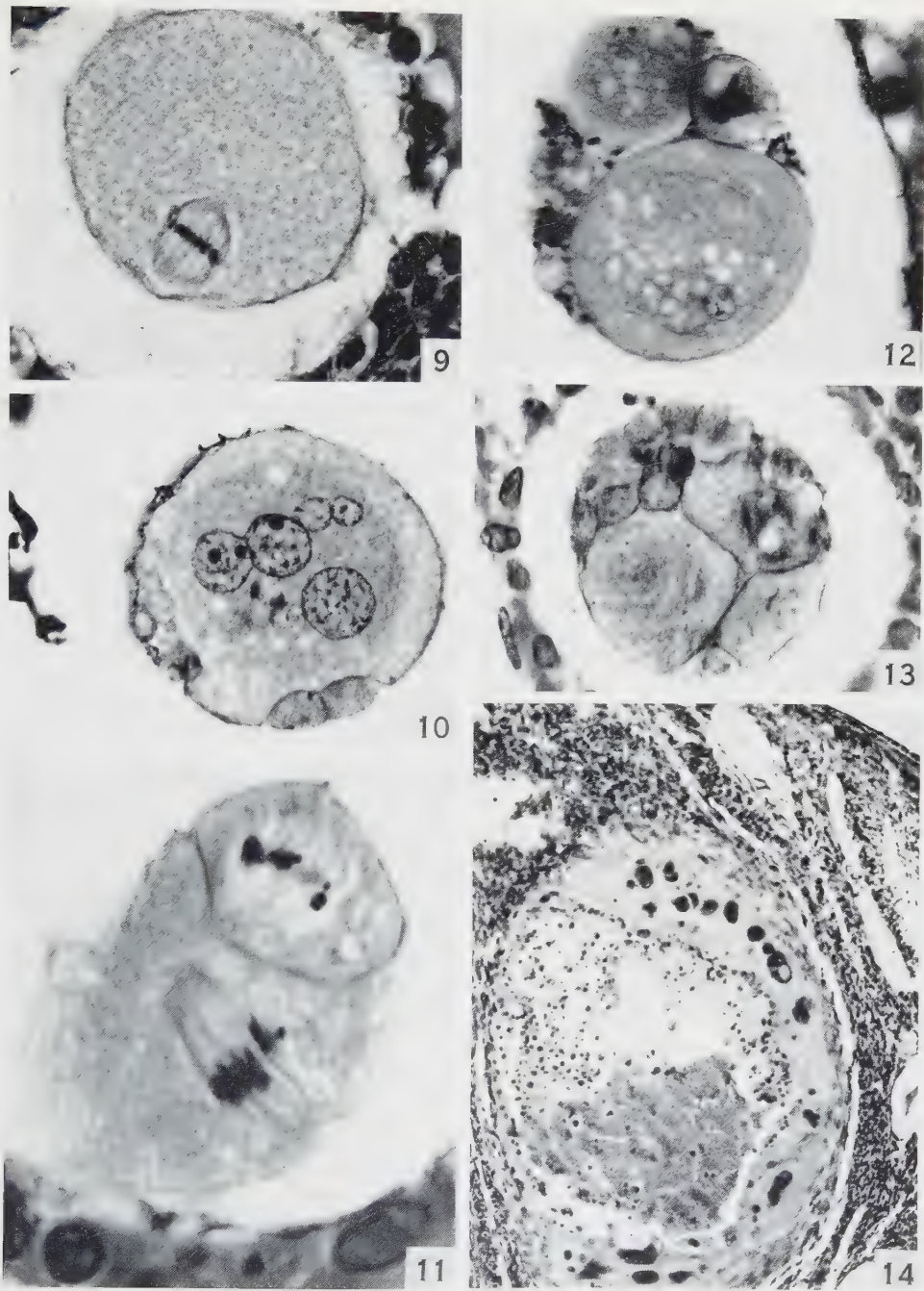
10 Multinucleate ovum with two polar bodies from an animal killed 26 hours after the injection of 40 R.U. pregnancy urine extract. Hematoxylin and eosin. $\times 725$.

11 Two-celled ovum in metaphase stage of second cleavage division, from a guinea pig killed 26 hours after the injection of 40 R.U. pregnancy urine extract. Hematoxylin and eosin. $\times 1100$.

12 Cleaving ovum from a guinea pig killed 26 hours after the injection of 40 R.U. pregnancy urine extract. Degeneration and resorption of this ovum are indicated by the infiltrated leucocytes which surround the egg. Hematoxylin and eosin. $\times 725$.

13 Morula stage from the ovary of a guinea pig killed 4 days after the injection of 25 R.U. pregnancy urine extract. Hematoxylin and eosin. $\times 725$.

14 Embryonic placentoma from the ovary of a normal animal killed at the beginning of heat. A giant cell layer, an inner cuboidal cell layer, and central hemorrhage may be observed. No decidual reaction in the ovarian stroma can be observed. Hematoxylin and eosin. $\times 60$.



THE MECHANISM OF KIDNEY DEVELOPMENT IN HUMAN EMBRYOS AS REVEALED BY AN EARLY STAGE IN THE AGENESIS OF THE URETERIC BUDS

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ONE PLATE (SIX FIGURES)

The examination of serial sections of an apparently normal human embryo of 9 mm. has revealed the failure of the kidneys, in this specimen, to develop. Because of the early stage of agenesis, an opportunity was thus presented for studying the role of different embryonic structures in the development of this comparatively frequent malformation.

The embryo in question had been fixed in Bouin's fluid and embedded in paraffin and no evidence of any anomaly had been suspected by gross inspection. Serial transverse sections of 8μ in thickness were made and stained after the azan modification of Mallory's connective tissue stain.

Upon section, the right umbilical artery was found to be abnormally small in diameter both in the umbilical cord and in the anterior abdominal wall; indeed it could not be traced to the aorta, its only visible connection being with an artery of the right leg. The corresponding artery on the other side is normal in size and position.

The right artery was probably undergoing obliteration at the time of death of the embryo. If this process could have gone on to completion, the artery in question might have become completely invisible in later stages and a primary occurrence of only one umbilical artery might have been predicated. This vessel would have differed only in position from

that in sireniform monsters in which both umbilical arteries are replaced by a single median vessel.

Among the organs of the urinary tract the mesonephros is wholly normal in structure on each side. Both wolffian ducts are present and of normal width ($10 \times 45 \mu$) in the mesonephric region. At the level of the caudal end of the mesonephros the ducts are dilated up to a diameter of about 100μ . It is difficult to say whether this is still within normal limits, as a slight dilatation always appears at this level. A short distance from the opening into the hind-gut the width of both ducts decreases considerably so that in the most caudal part a patent lumen cannot be seen with certainty on all sections. There is, however, no evidence of an obstruction. No ureteric buds can be found in this embryo. The only structure which possibly might be considered as a vestigial ureteric bud is a small dorsal diverticulum of the left wolffian duct originating from the dilated part shortly below the end of the mesonephros (U' in fig. 2). This formation, however, is hardly longer than wide, its length being about 40μ . No such structure can be seen on the right side.

The metanephrogenic tissue is very clearly visible on both sides. It is continuous with the mesonephros and forms a distinct cell mass on either side of the body. Figure 2 gives a view of this mass on the right side; in consequence of the curvature of the embryo the metanephrogenic cell masses of both sides cannot be shown in the same section. Both masses are clearly separated from each other by loose mesenchyma. The questionable vestigial left ureteric bud mentioned above does not reach the nephrogenic tissue. It ends at a distance of about 80μ from the latter.

This case affords an opportunity to study in a human embryo the appearance of the metanephrogenic tissue when not in contact with a ureteric bud. In chick embryos this condition has been produced experimentally by Boyden ('27, '32) and Grünwald ('37, '38 a) by damaging the growing end of the wolffian duct in younger stages. As a result, the ureteric bud failed to develop and the metanephrogenic tissue,

distinctly visible on the seventh day, could only be found with difficulty on the twelfth day of incubation, at a time when it should have developed distinct epithelial structures under normal conditions. In these experiments the differences in structure between the metanephrogenic cell masses with and without ureteric bud are quite similar to those to be described here (see below).

In the man, the youngest case hitherto described is a 10-mm. embryo (Boyden, '32). Besides a complete absence of the left ureteric bud, an abnormal contact between the two metanephrogenic masses was described and the question was raised whether this might not have led to the formation of a horseshoe kidney if both ureteric buds had been present. The histological structure of the tissue was not reported in detail because of the poor preservation of the embryo. However, the illustrations demonstrate clearly the great difference in size between the metanephrogenic tissue that is associated with a ureteric bud and that which is without a ureteric bud. Boyden emphasizes that there is no evidence that absence of a kidney is due to an aplasia of the metanephrogenic tissue. Gruber (l. c., p. 269) also deduces this from the fact that in cases of absence of one ureteric bud the other one may grow across the midline and form a kidney with the metanephrogenic tissue of the defective side.

Another case, much older however, was reported by Kornfeld in 1925-1926. This 5-cm. embryo has a defect that corresponds exactly to the condition that arises after experimental obstruction of the wolffian duct. The caudal part of the left mesonephros is missing as well as the wolffian duct, both at this level and caudal thereto. No kidney is present on this side and no trace of the metanephrogenic tissue is visible any longer, as I could confirm myself when Doctor Kornfeld kindly showed me the serial sections of his case.

In order to evaluate the influence of the wolffian duct on the metanephrogenic tissue, the kidneys of the present case have been compared with those of a normal embryo of the same stage, namely with a 9.5-mm. specimen, treated with the same

histological technic (figs. 3 and 5). A section of the cranial portion of the left mesonephros is given in figure 5. It shows a portion of the distal enlargement of the ureteric bud (U) in close contact with the metanephrogenic tissue (T). Farther cranial Schreiner's 'Zwischenblastem' is visible (Z), consisting of a small portion of the nephrogenic tissue which is not used for the formation of either mesonephros or metanephros. Figure 4 shows a portion of the left metanephrogenic tissue (T) of the abnormal embryo, which extends cranial up to the last tubule of the mesonephros (M). However, in this case the 'Zwischenblastem' cannot be differentiated from the metanephrogenic tissue. Both pictures are reproduced with the same magnification. Comparing the metanephrogenic tissues of both embryos, we find differences in number, shape, and arrangement of the cells. The difference in number is quite striking, as can be seen by comparing figures 2 and 3. Already, the metanephrogenic cells of the normal embryo must have undergone a rapid multiplication. The cells of the dense inner layer of the metanephrogenic tissue not only have oblong nuclei but are arranged radially around the ureteric bud. In the defective embryo where the ureteric buds are missing, the cells have round nuclei and are scattered irregularly. Only under low power does their arrangement suggest the presence of indistinct cell cords (fig. 2). There is thus a striking resemblance between the metanephrogenic tissue of this abnormal embryo and the 'Zwischenblastem' of normal embryos.

Similar conditions can be seen in the chick embryos used for the above mentioned experiments. In a 7-day embryo with no ureteric bud on the left side the left metanephrogenic tissue forms an indistinctly limited mass of cells with round nuclei differing from the connective tissue by the absence of fibers. In the normal kidney the cells have increased in number and are centered around the ureteric bud. There is a very large number of mitoses in the metanephrogenic tissue of the normal right side contrasting sharply with the rare incidence of cell division on the left side. Twelve-day embryos

of the same series of experiments show only small remnants of the metanephrogenic tissue on the side which had been operated upon. A low-power photograph of a section showing the difference between both sides was published previously (Grünwald, '37, fig. 5b). Figure 6 is a high-power view of another section of the left metanephrogenic tissue of the same embryo. There is a dense cell mass staining deeper with eosin than the surrounding connective tissue. Its nuclei are round in contrast to those of the connective tissue and no fibers can be seen in the nephrogenic cells. Otherwise no distinct border between the two tissues exists. In general the condition of the defective metanephrogenic tissue produced experimentally in the chick embryo is the same as that occurring spontaneously in the human embryo.

DISCUSSION

The above comparison of defective and normal human kidneys of the same age indicates clearly that contact with the ureteric bud is the factor that quickly induces multiplication, regular arrangement and changes in the shape of the metanephrogenic cells. The uninfluenced metanephrogenic tissue closely resembles in structure the small complex of undifferentiated nephrogenic tissue ('Zwischenblastem') which in normal embryos separates the blastema of the kidney from the mesonephros. This morphological similarity may be explained on the basis that these two tissues are developing under the same conditions, i.e., independently from the ureteric bud, in the abnormal embryo. This suggests that the 'Zwischenblastem' which disappears completely, is merely that portion of the metanephrogenic tissue which is uninfluenced by the ureteric bud.

Another point of interest is the normal 'migration' of the metanephros. Many explanations have been advanced both with respect to the way it happens and with regard to the role played by the two constituents of the kidney. As I pointed out in a critical review of different theories ('38 b), there are many unknown factors which may be concerned in

this change in position. Even at this late date we cannot say with assurance that the metanephrogenic tissue influences either the intensity or the direction of growth of the ureteric bud. The possibility that it does, has been emphasized especially by Gilman in his discussion of a rare form of dystopic kidney. Another theory, suggested by the course of normal development, is that the ureteric bud and its branches push the metanephrogenic tissue forward and thus accomplish the ascensus. This possibility received favorable consideration in my article of '38 b, but a study of this defective embryo shows that the distance between the metanephrogenic tissue and the point where the ureteric bud should originate, grows considerably within a short period even in absence of the ureteric bud. Apparently, therefore, this is merely a shift caused by relative differences in growth and therefore should not be ascribed to an action of the ureteric bud. Similarly, the subsequent change in position of the metanephrogenic tissue in respect to the mesonephros can be explained on the basis of the well-known downward transposition of the mesonephros and gonad. Moreover, the kidney by that time is so large compared with the diameter of the ureter that the latter could not possibly displace it by pushing it upward. This, however, does not mean that the ureteric bud is unable to influence the position of the kidney in early stages and thus to produce dystopia. It might cause, by irregular growth, a sufficient dislocation of the whole anlage to amount for the formation of a horse-shoe kidney, but all we can conclude from the present case is that the normal shift of the permanent kidney is due to differences in growth of the surrounding tissue and not to an active power exerted by the ureteric bud. Brockmann ('36) reaches the same conclusion although he failed to examine young stages. Starkenstein ('38), after investigating younger stages, maintained that the 'migration' of the kidney extends over a longer distance than is usually recognized. He opposes Brockmann's opinion and supposes an active ascensus, without giving any convincing reasons for it.

This rapid independent displacement of the metanephrogenic tissue observed in the present case, has another important bearing on kidney development. It predicates that the normal fusion of the two constituents which is essential for further normal development, can only take place during a short period, for the metanephrogenic tissue is withdrawn from the place where the ureteric bud should join it soon after the two are supposed to come together. It is doubtful whether a normal kidney can develop after that time, if one excepts the possibility that other cells can also be stimulated to kidney development by the ureteric bud. In this connection Brown's report is of special interest. This author investigated the embryonic development of a strain of mice which by earlier exposure to x-ray treatment had acquired high percentages of deformities of the urinary and other tracts. Some of these embryos had an unbranched ureteric bud reaching only to the border of the metanephrogenic tissue. The latter, however, showed no evidence of stimulation. Brown concluded: "It seems absolutely necessary that the ureter bud get into the blastema mass in order to form a functional kidney. Succeeding only in part or just reaching the blastema produces various conditions" (i.e., p. 147). To be sure, in Brown's cases the objection can be made that one or both of the renal primordia were rendered genetically inferior by the x-ray damage to the parents, but the anomaly can also be explained very well by retarded outgrowth of the ureteric bud.

Concerning the cause of agenesis in the present case, no evidence is available. Certainly, there is nothing to suggest a causal connection with the other two anomalies, the abortive right umbilical artery and the slight increase in diameter of the wolffian ducts.

Further development would certainly have led to complete absence of both kidneys, although it is possible that the rudimentary diverticulum of the left wolffian duct described above, might have formed an abortive ureter.

SUMMARY

1. The study of an early stage of aplasia of the ureteric buds in a 9-mm. human embryo shows that normally this structure influences the metanephrogenic tissue very soon after it comes in contact with it, thereby inducing the metanephrogenic cells to multiply and to assume the shape and arrangement typical for the normal internal layer of the metanephrogenic tissue.

2. The normal change in position of the permanent kidney is due to shifts by growth of the surrounding tissue and not to a pushing activity of the ureter.

3. It is probably of great importance that the ureteric bud grows out of the wolffian duct exactly at the correct time to warrant a normal development.

4. An early stage of obliteration of the right umbilical artery was noticed in the same embryo.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Section of the proximal part of the umbilical cord of the maldeveloped 9-mm. human embryo, showing the small diameter of the right umbilical artery (R) and the large left umbilical artery (L). C, coelomic cavity.

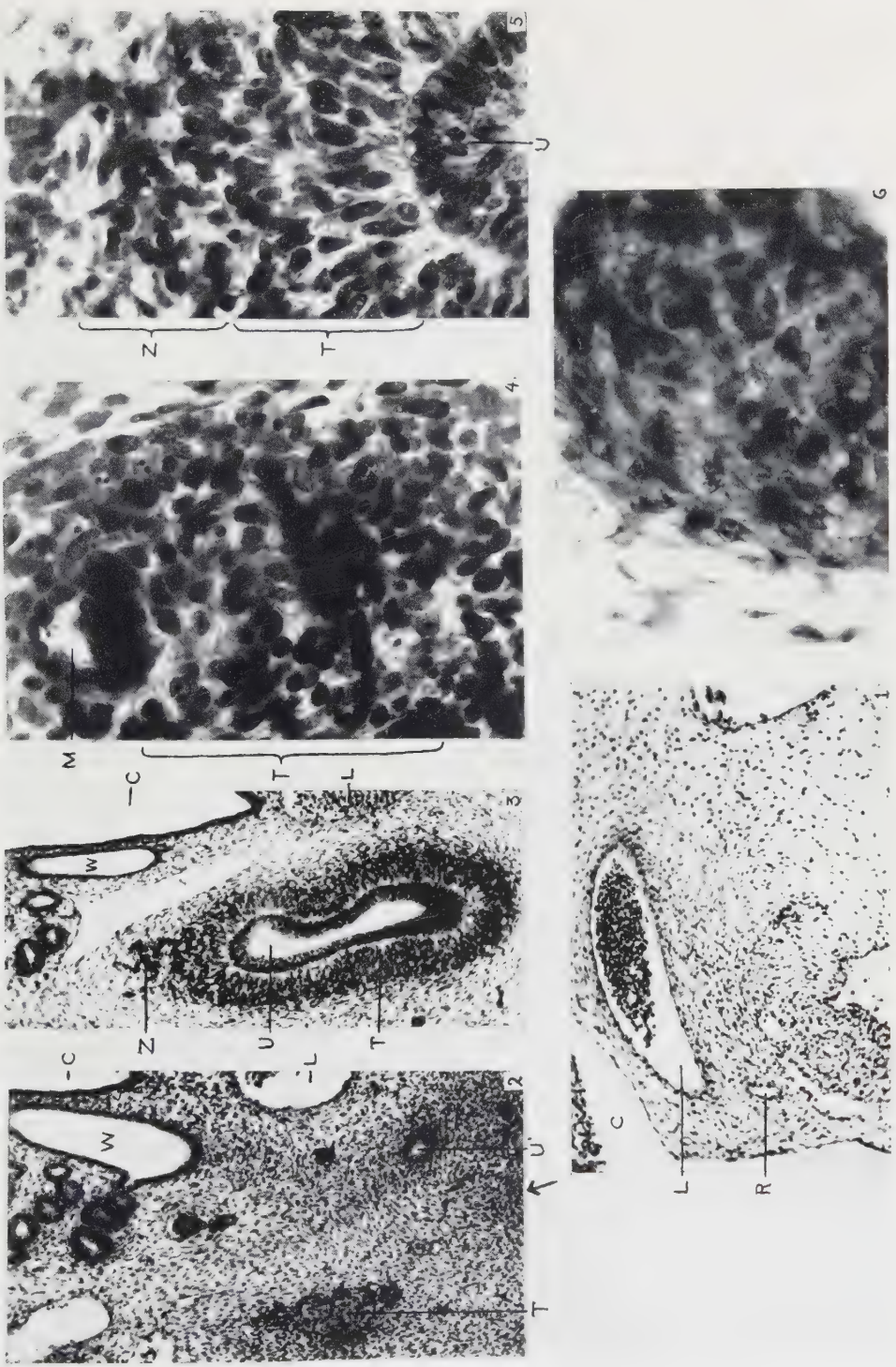
2 Frontal section of the same embryo, showing the right metanephrogenic tissue (T) which is not differentiating in the absence of the stimulating ureteric bud. C, abdominal cavity (left side); L, left umbilical artery; U', rudimentary left ureteric bud (?); W, left wolffian duct. Arrow indicates position of mid line.

3 Section of a normal 9.5-mm. human embryo, corresponding to figure 2. C, abdominal cavity; L, left umbilical artery; T, left metanephrogenic tissue; U, left ureteric bud; W, left wolffian duct; Z, 'Zwischenblastem.' Same magnification as in figure 2.

4 High power photomicrograph of the left metanephrogenic tissue of the same embryo as in figures 1 and 2. M, last mesonephric tubule; T, metanephrogenic tissue.

5 High power photomicrograph of a part of the normal kidney shown in figure 3. T, metanephrogenic tissue; U, ureteric bud; Z, 'Zwischenblastem.' Same magnification as in figure 4.

6 High power photomicrograph of the remnant of the left metanephrogenic tissue of chick embryo 438 (10 days and 20 hours of incubation), following destruction of the left wolffian duct on the second day of incubation and the consequent failure of the left ureteric bud.



STUDIES ON GONAD-HYPOPHYSEAL RELATIONSHIP AND CYCLIC OSSEOUS CHANGES IN THE ENGLISH SPARROW, *PASSER DOMESTICUS* L.¹

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ONE PLATE (SIX FIGURES)

INTRODUCTION

The marrow cavities of female pigeons contain spicules of bone if the ovary possesses follicles 10 mm. or more in diameter (Kyes and Potter, '34). Similar osseous changes followed the injection of estrogenic hormone into pigeons of both sexes (Pfeiffer and Gardner, '38). The writers observed that the bones of laying English sparrows are similarly hyperossified during the laying season. Since the sparrow is a seasonal breeder, but can be stimulated to complete gonadal activity during the non-breeding season (Kirschbaum, '33; Ivanova, '35; Kirschbaum and Ringoen, '36; Witschi and Keck, '35; Riley and Witschi, '37), it was felt that the osseous histogenetic changes might be studied to advantage by experimental stimulation of the gonads. The reproductive system of the sparrow was, therefore, stimulated by means known to

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² Alexander Brown Coxe Memorial Fellow in Anatomy (1937-1938).

³ Belgian-American Educational Foundation Fellow (1938-1939).

activate the reproductive mechanism during the non-breeding season. This led to interesting observations on the gonad-hypophyseal relationship in both the male and female sparrow, which are being reported at this time.

During the normal breeding season in the spring the male sparrow matures before the female. Using experimental procedures such as the injection of pregnant mare serum (Riley and Witschi, '38) and exposure to increased daily light ration (Ringeon and Kirschbaum, '39) it was observed that the male was more readily activated than the female. A crucial experiment was set up to determine whether this lag in female response is controlled by the hypophysis (Riley and Witschi, '37, '38) or whether the gonad itself is primary in the delayed ovarian stimulation.

It has been shown by Keck ('34) and confirmed by others that bill color in the sparrow is under hormonal control. Using the color of the bill as an indicator of male hormone secretion, further observations on androgen production in the sparrow are also being reported. Perry's ('38) hypothesis that the feeding of whole wheat grains previously irradiated with ultraviolet light will result in stimulation of the sparrow reproductive organs was tested.

MATERIALS AND METHODS

Nineteen juvenal and five adult female sparrows were injected with either 250 or 500 i.u. of estradiol-benzoate⁴ each day for from 3 to 29 days during May, June and July (table 1). Fifteen males received the same treatment. This experiment was undertaken to determine whether hyperossification of the bones might be produced, as had been observed by two of the authors in the case of pigeons. Four juvenal females, four juvenal males and two adult females were injected with pregnant mare serum at this time to determine whether the ovaries might be stimulated and a hyperossification of the bones obtained in this manner. The injected males were to

⁴ The estradiol benzoate was generously supplied by the Schering Corporation through the courtesy of Dr. E. Schwenk.

TABLE 1

Effects of estrogen and pregnant mare serum injections into female and male sparrows

NUMBER OF BIRDS	AGE	MATERIAL INJECTED DAILY	DATE INJECTIONS GIVEN	NUMBER OF DAYS INJECTED	CONDITION OF GONADS	CONDITION OF MARROW CAVITIES OF LONG BONES	REMARKS
Seven ♀	Juvenal	500 i.u. estrogen ¹	May-July	3-21	Inactive	Marrow only	Oviducts enlarged
Two ♂	Juvenal	500 i.u. estrogen	May-June	16-21	Inactive	Marrow only	
Five ♀	Adult	500 i.u. estrogen	May-June	2-23	Small follicles or inactive	Marrow only (2) Spongy bone (3)	Oviducts enlarged
Twelve ♀	Juvenal	250 i.u. estrogen ¹	June-July	10-29	Inactive	Marrow only	Oviducts enlarged
Thirteen ♂	Juvenal	250 i.u. estrogen	June-July	4-20	Inactive	Marrow only	
One ♂	Adult	2500 i.u. estrogen ¹	January	10	Inactive	Marrow only	
Eight ♀	Juvenal	2.5 r. u. mare serum ²	Oct.-Jan.	4-106	Inactive	Marrow only	
Two ♀	Juvenal	2.5 r. u. mare serum ²	Oct.-Apr.	176 & 199	Active	Marrow only	Darkening of bills. Follicles with yolk, but no ovulation
Ten ♀	Juvenal	2.5 r. u. mare serum	Dec.-Mar.	26-90	Inactive	Marrow only	Slight stimulation of ovary in a few
Two ♀	Juvenal	2.5 r. u. mare serum ³	Oct.-Mar.	101-124	Active	Marrow only	Follicles with yolk, but no ovulation.
Three ♀	Juvenal	2.5 r. u. mare serum ³	Jan.-Mar.	50-83	Active	2 with spongy bone	Darkening of bills Eggs in oviduct where cavities of long bones filled with spongy bone. Bills darkened
Two ♀	Adult	2.5 r. u. mare serum	May	10 & 13	Active	Spongy bone	Eggs in oviducts
Four ♀	Fledgling	2.5 r. u. mare serum	May	11-27	Inactive	Marrow only	
Four ♂	Fledgling	2.5 r. u. mare serum	May	16-27	Active	Marrow only	Testes averaged 5.5 mm. × 4.5 mm. Bills black
Two ♂	Juvenal	2.5 r. u. mare serum	Oct.-Dec.	20 & 37	Active	Marrow only	Testes fully active. Bills black
Seventeen ♀	Adult		Killed during study of ossification	May for	Active	5 birds had spongy bone in marrow cavities	Spongy bone in marrow cavities only when ovary at full activity—eggs usually in oviduct
Six ♂	Adult		Killed during study of ossification	May for	Active	Marrow only	

¹ Estradiol benzoate.² Gonadotropic hormone in whole serum.³ Ten r.u. purified extract used beginning March 3rd.

serve as controls. Six normal adult males and seventeen normal adult females trapped during the months of May and June were examined to determine the normal degree of long-bone ossification during the breeding season.

Twenty-five juvenal female sparrows were injected daily with 2.5 r.u. of pregnant mare serum (4 to 199 days) from October to April. A crude preparation was used previous to March 3rd, 10 r.u. of a purified extract⁵ subsequently. Two juvenal males were injected for 20 and 37 days as controls.

Beginning October 25th, ten juvenal males and ten juvenal females were exposed to 7 hours of added daily light (4 P.M. to 11 P.M.). Since Perry ('38) had reported gonad stimulation by feeding irradiated wheat, nine juvenal males and nine juvenal females were fed wheat which had been exposed to a Sperti Sunlamp⁶ for from 200 to 450 hours previous to feeding. Five males and five females were fed untreated whole wheat grains to control this experiment.

To determine whether the testis responds to secretion of the stimulated female hypophysis while the ovary is refractory to gonadotropic secretion, thirteen juvenal females received juvenal testicular grafts (table 2). Four operations were performed on December 20th, six on December 22nd and three on January 14th. Since three animals received second grafts (see table 2), a total of sixteen testicular grafts were made into intact females. In addition two incompletely spayed females received testicular grafts. Autoplastic testicular grafts were made into seven castrate males on December 20th, 22nd and January 14th (table 2). Three castrate and two unilaterally castrate males received ovarian grafts during this period. Grafts were made by making an incision between the two lowest ribs, spreading the ribs apart and dropping the fresh tissue into the peritoneal cavity.

⁵ The purified mare serum (gonadin) was supplied by the Cutter Laboratories through the courtesy of Dr. D. H. Wonder.

⁶ The authors are indebted to Mr. R. A. Lostro of the Science Laboratories, Inc., Cincinnati, Ohio, for his cooperation in making available a suitable supply of Sperti Sunlamps.

TABLE 2

Results of grafting juvenal sparrow testes into female and male sparrows exposed to increased daily light ration

NUMBER OF BIRD	DATES OPERATED	ORGANS EXTIRPATED	TISSUE GRAFTED	DATE KILLED	DAYS OF ADDED LIGHT	RESULTS
89 ♀	1/14/39	Ovary	Testis	2/11/39	28	Graft negative, ovarian fragment inactive
93 ♀	12/20/38		Testis	2/ 4/39	46	1 graft recovered (negative), ovary stimulated ¹
	1/24/39		Testis			
95 ♀	12/20/38		Testis	2/ 4/39	46	Graft recovered (negative), ovary stimulated
	1/24/39		Testis			Graft recovered (negative)
62 ♀	12/20/38		Testis	2/ 4/39	46	Graft recovered (negative), ovary stimulated
60 ♀	12/20/38	Ovary	Testis	2/ 4/39	46	Ovary stimulated, necrotic graft removed (1)
	1/24/38					
22 ♀	12/22/38		Testis	2/11/39	51	Enlarged testicular grafts (2), ovary negative
	1/24/39					
64 ♀	12/22/38		Testis	2/ 3/39	43	Graft negative, ovary stimulated
65 ♀	12/22/38		Testis	1/27/39	36	Enlarged testicular graft, ovary negative
69 ♀	12/22/38		Testis	2/ 3/39	43	No graft recovered, ovary stimulated
74 ♀	12/22/38		Testis	2/20/39	60	No graft recovered, ovary stimulated
76 ♀	12/22/38		Testis	2/ 4/39	44	Enlarged testicular graft, ovary negative
79 ♀	1/14/39		Testis	2/20/39	37	No graft recovered, ovary stimulated
80 ♀	1/14/39		Testis	2/11/39	28	No graft recovered, ovary negative
86 ♀	1/14/39		Testis	2/20/39	37	No graft recovered, ovary negative
61 ♂	12/20/38	Testes	Autoplastic testis	2/28/39	70	Enlarged testicular graft
71 ♂	12/22/38	Testes	Autoplastic testis	2/22/39	62	Enlarged testicular graft
72 ♂	12/22/38	Testes	Autoplastic testis	2/ 3/39	43	Slight growth of testicular graft
75 ♂	12/22/38	Testes	Autoplastic testis	2/ 4/39	44	Graft negative
77 ♂	12/22/38	Testes	Autoplastic testis	2/ 4/39	44	Slight growth of testicular graft
78 ♂	1/14/39	Testes	Autoplastic testis	2/11/39	28	Enlarged testicular graft
87 ♂	1/14/38	Testes	Autoplastic testis	2/11/39	28	Enlarged testicular graft

¹ In all instances where ovarian stimulation is recorded, such hypertrophy was slight with follicles usually 1 mm. or less in diameter.

All injections of hormone were made into the breast muscles of the sparrows, a 27-gauge needle being used. Bones were fixed in formalin, decalcified, embedded in paraffin, sectioned at 10μ and stained with Dominici's combination of eosin-orange G and toluidin-blue. Other tissues were fixed in Bouin's fluid and stained with Delafield's hematoxylin. All birds were kept in cages $3 \times 3 \times 5$ feet, an average of fifteen birds being kept in one cage. A diet consisting of equal parts of millet seed and growing chick mash was fed the birds. Water was present at all times.

The Sperti Ultraviolet Sunlamp was used as the source of light in supplying added daily light ration. One lamp, backed by a reflector, provided light for one cage. Wheat was irradiated by spreading the grains in a single layer under a battery of Sperti Sunlamps, the arrangement being such that all wheat was an average distance of 1 foot from the source of light.

OBSERVATIONS

The bones of adult female sparrows which are laying eggs during May were found to contain osseous spicules in the marrow cavities (fig. 3). Females trapped with their young at the time the latter leave the nest did not show this condition. Unless the ovary was near maximal activity or eggs were found in the oviducts, hyperossification of the long bones did not occur. Injections of estradiol benzoate did not bring about similar osseous changes in the bones of juvenal males and females. Three out of five adult females of uncertain state of breeding cycle (table 1) injected with this estrogenic substance during May and June had spicules of bone in the marrow cavities.

The ovaries of juvenal females could not be stimulated to full activity by the gonadotropic substance (2.5 r.u. daily) in mare serum between the months of May and March. Full ovarian activity was observed, however, during March and early April in five birds injected with pregnant mare serum. The bones of two of these were hyperossified (fig. 2) and eggs were present in the oviducts of both females (fig. 4).

The hyperossification was not as extensive, however, as that found in some laying females from nature. The testes of juvenal males are apparently capable of responding to gonadotropic substance at any time after hatching. Complete spermatogenic activity was induced by injections of pregnant mare serum during May and anytime thereafter.

The ovary was not appreciably stimulated when birds were exposed to added light after dark (4 P.M. to 11 P.M.) during October, November and December. The testis, on the other hand, could be fully stimulated. Full ovarian activity (ovulation) was not observed at any time as the result of exposing the birds to added daily light. Although the oviduct enlarged significantly in those experiments run from December 20th to February 20th, maximal oviduct size was not obtained. The bones of these light-stimulated females did not contain osseous spicules in the marrow cavities.

The feeding of whole wheat grains previously irradiated by rays from the Sperti Sunlamp proved ineffective in stimulating either the male or female reproductive apparatus.

Testes grafted into females enlarged significantly (fig. 5) in four instances when the hosts were exposed to added daily light (table 2); microscopically the germinal epithelium was spermatogenically active (fig. 6). The ovaries of birds with positive testicular grafts were inactive (fig. 5). Grafted testicular tissue was recovered in nine of the eighteen operations. In five cases mitoses were not found in the germinal epithelium. In these latter cases, intertubular lymphoid tissue was hyperplastic. Mitotic divisions were observed in some lymphocytes. True Leydig cells could not be identified. In all but two of those instances where grafts were not recovered slight ovarian stimulation was evident, whereas in those instances where grafts were stimulated to growth the ovary was inactive. Only one out of five ovarian grafts into males was recovered, and this was inactive. Of seven autoplasmic testicular grafts all were recovered, six being enlarged.

In each instance where the ovary enlarged as a result of injecting pregnant mare serum, pronounced darkening of the

lower as well as the upper half of the bill was noted. Normal females show a similar bill-color change during the breeding season. In no case did the female bill become as black as that of the male with active testes. The bills of neither males nor females darkened as a result of injection of estrogenic substance.

DISCUSSION

The fact that hyperossification of the long bones is not very evident unless an egg is found in the oviduct or the female sparrow has already laid eggs indicates a close correlation with high ovarian activity and possibly estrogen production. However, contrary to the findings on pigeons (Pfeiffer and Gardner, '38) the dosage of estrogens used in this experiment failed to achieve such results in the sparrow. It is possible that the dosage was too high since there is some evidence (unpublished data on pigeons) that high doses are not as effective as appropriate lower doses. The one instance (see table 1) where an extremely high dose of estrogen was administered would seem to preclude the possibility that insufficient quantities of estrogen were given. The mechanism in the sparrow might, possibly, differ from that in the pigeon. Although adult females injected with estradiol benzoate during May showed little ovarian activity and hyperossified bones, the condition of the bones at the time treatment was begun was not known.

The fact that spicules of bone were found in the long bones when the ovary was stimulated to full activity during the non-breeding season by injection of pregnant mare serum proves that hyperossification of the long bones represents a seasonal cyclic physiological change in the female sparrow dependent on seasonal ovarian activity. These females were autopsied upon the appearance of the first egg in the oviduct. The degree of ossification (fig. 2) was not as great as that in the laying females trapped during May and June (fig. 3). This may be due to the fact that the latter had laid several eggs and therefore had been subjected to a more prolonged or intense ovarian stimulation. These bone changes probably

occur very rapidly since only a few days elapse between the appearance of the first and last eggs of a clutch in the oviduct. Since females trapped with their young at the time the latter leave the nest had no bony spicules in the marrow cavities (fig. 1), it is felt that the phenomenon in question is rapidly reversible as well as quickly induced.

It is interesting to note that the ovary was not refractory to gonadotropic hormone after 150 daily injections (October to March). Although no marked gonad response was exhibited during this period, when a purified extract was injected (see table 1) fully active ovaries were obtained within 17 days. The juvenal testis responded readily (within 15 days) to the same material which failed to substantially stimulate the juvenal ovary. It is possible that the ovaries might have become refractory because of the associated protein in unfractionated serum. They were not, however, refractory to the hormone itself as indicated by the response of the gonads. It is just as logical to assume that the ovaries responded at this time because of the proximity to the normal breeding season. Sparrows have likewise failed to become refractory to repeated injections of thyrotropic hormone (Miller, '38).

Since testes are more readily activated than ovaries by injections of gonadotropic hormone (Witschi, '35; Riley and Witschi, '37, '38) and by exposure of birds to increased daily light ration (Ringoen and Kirschbaum, '37, '39; Riley and Witschi, '37, '38), the problem had arisen as to whether the differential in response is inherent in the hypophysis or the gonads. Riley and Witschi ('38) felt that "the ineffectiveness of additional daily light period in the production of an extensive ovarian development indicates a low degree of responsiveness of the female pituitary to light." It is further stated that "whether the negative reactions given by some of the injected females (with gonadotropic hormone) in fall and winter is due to a refractoriness of the gonad or to the absence of essential supplementary activity by the birds' own pituitary, or even to the production of an inhibitory substance during

the quiescent period is not cleared up by these (their) experiments.”

Our observations indicate that the testis can be stimulated when grafted into females exposed to added daily light. The testis is then under the influence of the secretions of the female hypophysis, and is activated when the animals' own ovaries show no stimulation. Although Pfeiffer ('36) has shown in the rat that under certain conditions the testicular graft can masculinize the hypophysis of the female host, the age of the birds and the short period of time that the grafted testes were present in the females would argue against such an interpretation here. If the ovary inhibits the hypophysis, such inhibition is not effective in preventing testicular development. It would seem, then, that the difference in response of testis and ovary is primary in the gonads themselves. Since in this experiment the testicular grafts into males were autotransplants, it is impossible at this time to state that the female hypophysis is stimulated to the same extent as that of the male. Although in this experiment testis transplants 'took' better in the male, this might be explained on the basis of the fact that they were autotransplants in the case of the male (homotransplants in the female). This phase of the problem is being investigated further.

Bill color is a secondary sex character in the sparrow. The bill of the male is black during the breeding season. Castration results in loss of pigment which can be restored by injection of androgen (Keck, '34). Keck also showed that the female bill responds to injections of male sex hormone. Injection of estrogen alone does not cause a similar change. The female bill darkens during the breeding season, but not to the same extent as that of the male. This darkening of the female bill has not previously been attributed to secretion of male hormone by the ovary. When the ovary was activated by injections of pregnant mare serum under laboratory conditions, it was noted that the bill darkened to the same degree as during the breeding season. Since androgenic substance is the only material known at present to influence bill coloration

in this manner, it must be concluded that the active ovary secretes appreciable amounts of male sex hormone.

In the present experiment the contention of Perry ('38) that the feeding of whole wheat grains previously irradiated with the rays of the Sperti Sunlamp induces precocious gonad development in the male and female sparrow could not be confirmed. Perry's results must be attributed to factors which were not present in this investigation.⁷

CONCLUSIONS

1. Seasonal hyperossification of the skeletal system of the female sparrow can be correlated with seasonal ovarian activity. Although the bone changes have not thus far been duplicated with injections of estrogens, complete activation of the ovary during the non-breeding season was accompanied by the laying down of osseous spicules in the marrow cavities.

2. Juvenal sparrow testes grafted into females exposed to added daily light ration in the winter were stimulated to produce spermatozoa. Gonadotropic secretion of the female hypophysis failed to stimulate the hosts' own ovaries, whereas the grafted testes were fully stimulated. The testis was more responsive than the ovary to gonadotropic secretion.

3. The sparrow ovary secretes male hormone as indicated by darkening of the female bill during the normal breeding season and experimentally when the ovary was stimulated to full activity out of season.

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⁷In personal correspondence Doctor Perry states that the technique employed in this investigation was similar to his. Factors such as the length of time the wheat had been stored, conditions under which the grain was grown and stored might explain the discrepancy in results, according to Doctor Perry.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

- 1 Section through the shaft of the tibia of a sexually immature female sparrow. Fatty marrow with islands of hemopoietic tissue. Bones of males show a similar picture whether the bird is sexually active or inactive. $\times 150$.
- 2 Similar section from a female whose ovaries had been activated with gonadotropic hormone. An egg was present in the oviduct (fig. 4). Spicules of partially ossified new bone present in the marrow cavity. $\times 90$.
- 3 Similar section from a laying female trapped during May. An egg was present in the oviduct. Bony spicules in the marrow cavity; more complete ossification of spicules than in figure 2. $\times 150$.
- 4 Reproductive tract of female sparrow which received injections of pregnant mare serum during the non-breeding season (October to March). Ovary active, speckled egg in oviduct. Approximately natural size.
- 5 Testicular graft in a female host which had been exposed to added daily light from December 20th to February 4th. Testicular graft (t) enlarged, host's ovary(o) and oviduct (ov) of minimal size. Slightly larger than natural size.
- 6 Section of testicular graft shown in figure 5. Active germinal epithelium, spermatozoa present. $\times 200$.



THE ACTION OF ESTROGENS IN INDUCING MITOSES IN THE MUSCLE, CONNECTIVE TISSUE AND EPITHELIUM OF THE PROSTATE AND SEMINAL VESICLE OF MICE AS DETERMINED BY THE COLCHICINE TECHNIQUE

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ONE PLATE (TWO FIGURES)

The Amsterdam group of workers ('33, '34, '35, '38), Lacasagne ('33), Burrows and Kennaway ('34, '35), Korenchevsky and Dennison ('34) have described the effect of estrogens on the secondary sex glands of male mice. The principal effects of such treatment are an increase in the fibro-muscular stroma, stratification and cornification of the epithelium in the dorsal prostatic lobes. The latter effect is analogous to the cornification of the vagina occurring during estrus. As treatment continues these changes are followed by similar effects in the seminal vesicles, ejaculatory ducts, vasa deferentia, the other lobes of the prostate, the urethra and the urethral glands. The effects of estrogens on other male mammals have also been investigated, by Overholser and Nelson ('34, '35) in the rat, by Weller, Overholser and Nelson ('36) in the rat and mouse, by Wells ('36) in the ground squirrel, by de Jongh and Kok ('35) in the dog, by van der Woerd ('35, '36) in the guinea pig, by Parkes and Zuckerman ('35, '36), Courier and Gros ('35), van Wagenen ('35) in various species of monkeys. In general no mitoses in the fibro-muscular stroma

¹ The present work was begun at the Pharmacotherapeutic Institute, University of Amsterdam.

were described. Fleischmann and Kann ('38) using the colchicine method observed mitoses in the epithelium of the seminal vesicle after estrogen treatment.

Allen, Smith and Reynolds ('37) found by the aid of colchicine that the muscles of the uterus of the ovariectomized mouse and rat undergo hyperplasia in response to ovarian follicular hormone (theelin). The uterus of the ovariectomized rabbit, distended with paraffin pellets, but not treated with sex hormones, also showed hyperplasia at the sites of distention. Therefore apparently both endocrine stimulation and distention are factors in hyperplasia of uterine muscles.

In the present investigation we attempted to study by the use of colchicine the effects of estrogens on the epithelium, muscle and connective tissue of the prostate and seminal vesicle of castrated mice.

MATERIAL AND METHODS

Infantile male albino mice,² 2 weeks of age, were castrated and injected with estrogens. After treatment with estrogens for 1 week, the animals were injected with colchicine, 2 γ per gram of body weight and sacrificed 8 hours later. The seminal vesicles and prostate were fixed in Bouin's fluid, embedded in paraffin and stained with iron haematoxylin and orange G, or with haematoxylin and eosin.

RESULTS

In the animals of the first series treatment with estradiol benzoate and diethyl-stilboestrol was begun 4 weeks after castration. Five animals received four injections of 20 γ estradiol benzoate, five received 100 γ estradiol benzoate per injection, ten other animals received corresponding doses of diethyl-stilboestrol. The material was dissolved in olive oil in such concentration that 0.1 cc. contained the dosage to be used. The injections were given on the twenty-eighth, twenty-ninth, thirty-third and thirty-fifth day after castration. On

²The animals were generously supplied by Dr. Margaret R. Lewis and Dr E. Laqueur.

the thirty-sixth day the animals received colchicine and were sacrificed 8 hours subsequently (table 1).

In a second series of experiments the animals were injected on the fifth and twelfth day after castration with 100 γ estradiol di-propionate and on the fourteenth day they were treated with colchicine and sacrificed (compare table 1).

TABLE 1

NUMBER OF ANIMALS	SUBSTANCE INJECTED AND TOTAL AMOUNT (IN GAMMA)	MITOSES IN:			
		Fibro-muscular stroma		Epithelium	
		Prostate	Seminal vesicle	Prostate	Seminal vesicle
10	Controls with colchicine	No mitoses	No mitoses	No mitoses or at most 1 or 2 mitoses	
5	Estradiol benzoate				
	80	+	+	+	+ ¹
5	Diethylstilb- oestrol				
	80	+	+	+	+
5	Estradiol benzoate				
	400	+	+	+	+
5	Diethylstilb- oestrol				
	400	+	+	+	+
4	Controls with colchicine	No mitoses	No mitoses	No mitoses or at most 1 or 2 mitoses	
4	Estradiol di- propionate, 200	+	+	+	+

¹ + means more than 5 mitoses per field ($\times 280$).

The controls, injected with colchicine alone, showed no mitoses in muscle or connective tissue cells and only rare mitoses in the epithelium of the seminal vesicles or prostate (1 or 2 mitoses per field, $\times 280$). On the other hand, the animals treated with estrogens showed numerous mitoses in the muscle and connective tissue surrounding the seminal vesicles (compare fig. 1) and the prostate (compare fig. 2) as well as some mitoses in the epithelium of the prostate and seminal vesicle.

The epithelium of the prostate showed the well-known stratification and cornification with mitoses in the basal cell layer. The mitoses in the epithelium of the seminal vesicles were more numerous than in the controls, often as many as ten to twelve per field ($\times 280$).

It is easy to distinguish between the various tissue elements and to differentiate the mitoses of the epithelium, muscle, or connective tissue, as the basal membrane and the muscle stain deeper with orange G than do the epithelium or connective tissue.

Mitoses were observed also in the epithelium of the seminal vesicles of animals treated with estrogens, 5 or even 28 days after castration. It is possible that in the series treated 5 days after castration, the estrogen acted, as suggested by Freud, as a 'pace maker' for androgens still circulating in the body. However, the results obtained in the series of animals, treated with estrogens 28 days after castration, prove that the estrogens may induce mitotic activity by their direct action also or by stimulation of other organs to produce an androgen.

In testing the presumed androgenic action of a given extract by its capacity to induce mitotic activity in the secondary male reproductive system it is important to remember that any estrogen which such extracts might contain may have the effect described above. With the method described, the occurrence of stratification and cornification of the prostatic epithelium and of mitoses in muscle and connective tissue indicates the presence of an estrogen, while mitoses in the epithelium of the seminal vesicles indicate the presence of either an androgen or estrogen. The effect on the epithelium induced by an androgen is greater, however, than that induced by a comparable dose of the estrogen. The number of mitoses in the fibro-muscular stroma and in the epithelium of the prostate and seminal vesicle in the several animals treated with estrogens is variable. Therefore the present observation seems to be applicable for a qualitative rather than a quantitative test.

SUMMARY

The effect of estrogens on the fibro-muscular stroma of the seminal vesicle and of the prostate of animals is readily observed by the aid of colchicine. Some mitotic figures are found also in the epithelium. The difficulty inherent in testing a mixture of androgens and estrogens, is pointed out and a method is suggested for a qualitative distinction between the effect of these substances.

An artificial estrogen, diethyl-stilboestrol, produces changes in the prostate and seminal vesicle similar to those induced by estrogens with steroid structure.

I wish to express my thanks to Dr. C. G. Hartman for his interest and help.

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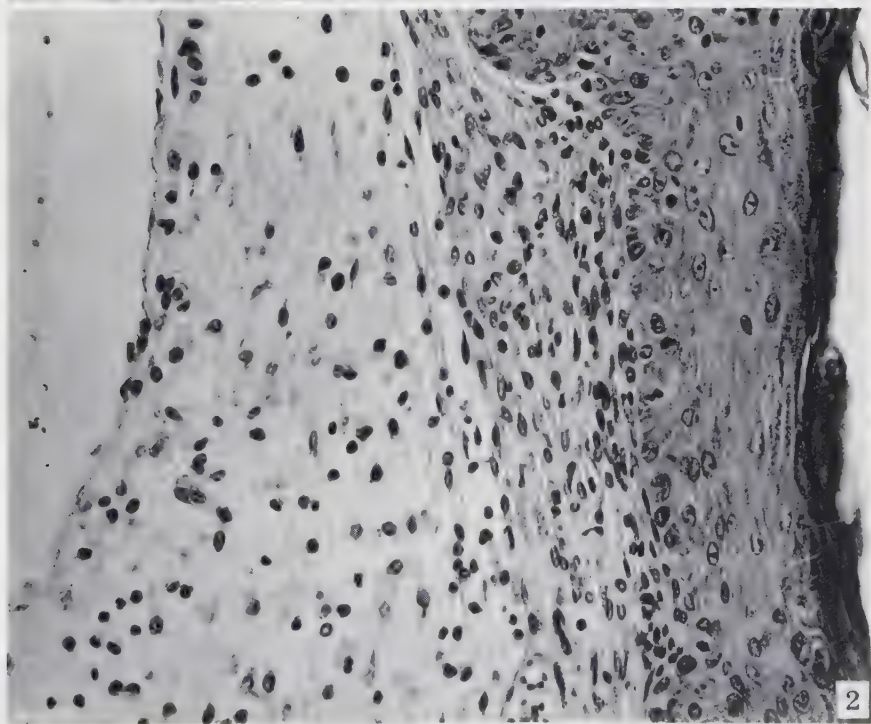
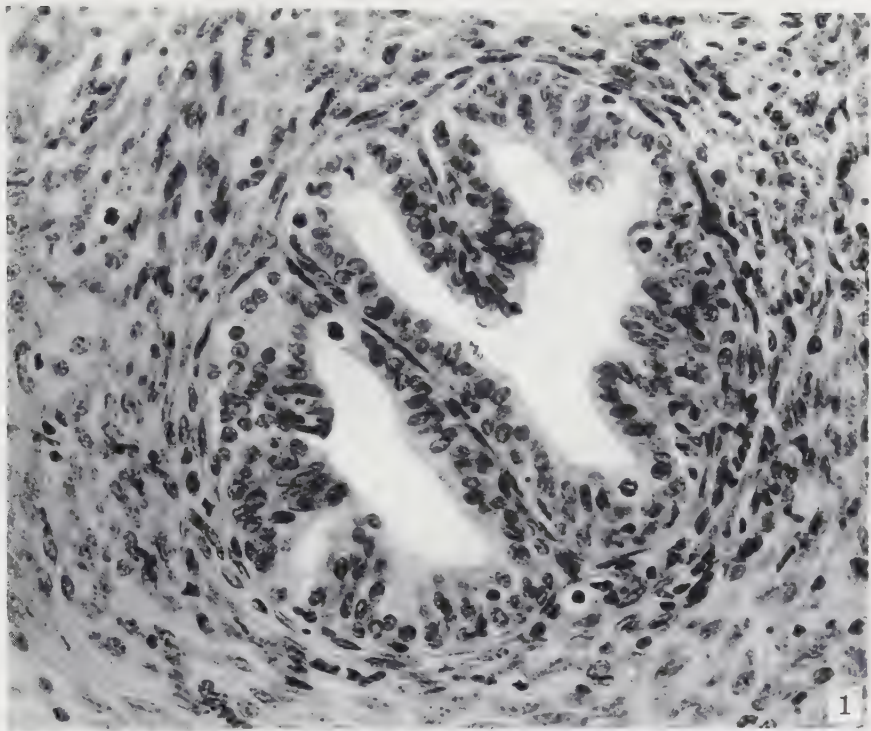
PLATE

PLATE 1

EXPLANATION OF FIGURES

1 The seminal vesicle of an infantile castrated mouse injected with 200 γ estradiol di-propionate. Mitoses in the fibro-muscular stroma accumulated and arrested by the aid of colchicine. $\times 400$.

2 The prostatic gland of an infantile castrated mouse injected with 200 γ estradiol di-propionate. Mitoses in the basal cell layer of the epithelium, in the smooth muscle and connective tissue, arrested by the aid of colchicine. $\times 300$.



EPIDERMAL CONCENTRIC CORPUSCLES AND THEIR SIGNIFICANCE IN THE INTERPRE- TATION OF THE CORPUSCLES OF HASSALL: TRITURUS

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ONE PLATE (EIGHT FIGURES)

INTRODUCTION

Opinion concerning the nature and significance of Hassall's corpuscles has tended to crystallize about two interpretations which appear most worthy of consideration in the light of our present adequate knowledge of the histogenesis of, and meager knowledge of the function of, the thymus.

One of these interpretations is that these corpuscles represent segments of thymic blood vessels, the lumina of which have undergone local occlusion as a result of endothelial cell hypertrophy occurring in the course of normal or pathological involution. Assuming this interpretation to be correct, and believing it should be possible, therefore, to demonstrate comparable structures arising from blood vessels in other lymphoid tissue in the process of involution, Jordan and Horsley ('27) examined certain involuting subcutaneous and abdominal lymph nodes of the rabbit. In these organs they found bodies which they homologized with the thymic corpuscles of Hassall, and which they believed arose from medullary capillaries and precapillary arterioles. Afanssiew (1877) supported the theory of the vascular origin of the corpuscles, but held the vessels involved to be veins. Hemmeter ('26) describes the occurrence of Hassall's corpuscles within the spleen of a shark, *Alopias vulpes*, and intimates, but does not definitely state, that these bodies arise by proliferation of the endothelium.

The opposing interpretation considers these corpuscles to be derived from the epithelial constituents of the organ. They may represent regressive remnants of the epithelial anlage of the thymus which have accumulated in characteristic concentric bodies; or they may be expressions of the growth and differentiation of an active epithelium, the ultimate potentialities of which have been retained, but the development of which has been modified by the circumstances of the unfavorable environment (Dearth, '28). Kingsbury ('28), in support of the latter conclusion, sets forth a number of salient facts concerning the histogenesis and histology of the thymus and suggests several potent arguments in opposition to the interpretation of the corpuscles as of vascular origin. Juba and Mihálik ('29) likewise describe them as being of (epitheloid) cytotreticular origin. Norris ('38) presents embryological evidence of the derivation of these bodies from certain cells of the cortex, which he shows to have been derived from a portion of the cervical sinus, an ectodermal structure.

Kostowiecki ('30) maintains that the corpuscles have a double origin, their formation being initiated by hypertrophy of epitheloid cells which assume a concentric arrangement but growth in diameter being due, at least in part, to encroachment of the epitheloid complex on nearby involuting veins, which then enter into the formative corpuscle at the periphery. Thus, the corpuscle is considered to be a composite structure.

Hammar ('21, '24, '27) has attributed to the corpuscles a physiological significance, believing them to be sources of antitoxic activity.

It is the purpose of this paper to describe certain concentric corpuscles produced experimentally in tongues of epidermal tissue mechanically invaginated during subcutaneous implantation of pituitary glands in *Triturus*. The development of these corpuscles will be described and their relationship to Hassall's corpuscles and to epithelial pearls will be discussed.

METHOD AND MATERIAL

The material employed in this study consisted of the implantation sites from pituitary-implanted *Triturus viridescens* eft's. Implants were made in the dorsal musculature by puncturing the skin and muscle with a sterile needle, and introducing the foreign tissue at an acute angle, well into the wound. In this process some of the surface epithelium was unavoidably carried into the site where it remained either attached to the original surface epithelium by a cord of epitheloid cells, or detached and isolated in active epitheloid nests. The age of the implant at removal of the site varied between 4 and 23 days. The tissue was fixed in Allen's B-15 fixative, sectioned 10 μ thick, and stained with Harris' hematoxylin and with eosin for routine inspection.

OBSERVATIONS

Examination of serial sections of the implant area reveals the presence of tongues of epitheloid tissue in a majority of the sites, appearing as invaginations of the thickened epidermal covering of the wound (figs. 1 and 2). In many cases the connection between the invaginated tongue and the superficial epidermis has been mechanically ruptured, and the epidermal cells have been isolated in the implant site (figs. 3 and 4). In addition to this epitheloid tissue, the immediate site is occupied by the implant, by ingrown mesenchyme, by collagenous fibers and dedifferentiating striated muscle elements, by blastema cells, varying numbers of melanin granules introduced by the needle, and by ingrown blood vessels of varying sizes, in accordance with the extent of degeneration and regeneration, the condition of the implanted tissue, and, in general, with the implant age. In this environment of connective tissue and blood vessels the epitheloid cells have remained alive and active, bearing the same approximate morphological relationship to each other as in the epidermis, modified, of necessity, to conform to the available space. Where surrounding tissues press closely upon the epitheloid nests the contours of the latter have tended to round off (figs.

3 and 4). In those instances where sufficient space has been available the tongues or nests retain a jagged outline indicative of the serration to which they were subjected by the needle. That these isolated nests are truly epitheloid is evinced by the shape and size of the cell and of the nucleus, by the compactness of the tissue, by the nature of the staining reaction, and especially by the presence, in many of the cells, of keratohyaline granules. It is in these epithelial nests that the concentric corpuscles have been located.

These corpuscles vary in size between $30\ \mu$ and $40\ \mu$. The smallest recognizable corpuscle is composed of a lumen, containing a large, squamous, epitheloid cell, and of one or two concentric layers of keratinized cells surrounding the lumen (figs. 5 and 6). The central cell has an irregular, cytoplasmic boundary, and a glassy, structureless nucleus, and is oriented so as to occupy a large cross section of the lumen of the formative corpuscle. No red blood cells have been found in any of the lumina. The cells of the concentric layers immediately surrounding the lumen are greatly flattened, partially desquamative, glassy cells with compressed, structureless nuclei, and homogeneous cytoplasm. Those of the next concentric layer contain a maximum number of keratohyaline granules, the nuclei are less compressed, contain one or two clumps of darkly staining material, and the cytoplasm is normal in appearance. The remaining cells in the immediate vicinity of the corpuscle have assumed a position with relation to the corpuscle such as to suggest a reorientation on their part, to conform to the concentric arrangement of the more central layers. No changes are yet evident in their protoplasmic constitution. The nucleus at this stage generally contains two prominent nucleoli.

As the corpuscle grows by addition of successive layers of squamous cells at the periphery, additional cells are added to the lumen by the sloughing off of the keratinized cells of the innermost concentric layer. These sloughed-off cells, together with the original lumen cell, compose a compact mass

of dead cells the nature of which, at this time, may be made out with difficulty. No red blood cells may be seen in the lumen at any time. At this more advanced stage, the numerous deep, concentric layers are composed of pale, wavy strands of keratinized, squamous cells (fig. 7). The hyaline granules, abundant in earlier stages, have dissolved to form the keratin of the cells of the deeper concentric layers. Only the intermediate layers now contain granules.

Separating these lamellae are concentric spaces formed by the shrinking of the cells on keratinizing. The outermost layers of the corpuscle contain epidermal cells with large numbers of keratohyaline granules. The nuclei may contain one or two prominent nucleoli, or they may be slightly shrunken from the adjacent cytoplasm, and almost homogeneous in appearance, with only a suggestion of the former nucleoli remaining. The nucleus does not decrease in size proportionally with the cytoplasm, which eventually becomes very much flattened, and the nuclei therefore cause the cells to bulge on all surfaces as keratinization progresses. Thus, the larger corpuscles differ from the smaller ones only in the increased number of concentric layers of squamous, keratinized cells, and in the more compact nature of the additional contents of the lumina.

Study of other areas of the implant site reveals the presence of additional bodies which appear to be immature corpuscles (fig. 8). Careful observation proves them to be small blood vessels the walls of which are markedly thickened, either as a result of local concentrations of connective tissue reticulum, or by hypertrophy of the endothelial cells, or both. The lumen of some of these is quite occluded and may contain two or three red blood cells and an occasional polymorphonuclear leucocyte. Other structures, in addition to blood vessels, which may be construed as concentric corpuscles, are present in the sections, but these lie immediately beneath the epidermis, and represent formative mucous glands.

DISCUSSION

The concentric corpuscles herein described agree in detail with descriptions of epithelial pearls as found in the pathological condition of epidermoid carcinoma, or plexiform epithelioma. The tissue under consideration, however, presents no evidence of hyperplasia or metaplasia. On the contrary, a condition exists wherein a normal, active, stratified epithelium has been mechanically invaginated. The epithelium in this instance is epidermal, and the cells thereof are specialized to differentiate, under the proper stimulus, to form squamous, keratinized cells, destined eventually to be sloughed off the epidermal surface. The mechanism underlying the phenomenon of differentiation is not understood, although in the ultimate analysis it must be referred to some function of the inherent protoplasmic constitution of the cell. The nature of the environment in which the differentiating cell exists, however, must, at least, be such as not to prevent differentiation from progressing. In order that epidermal cells may differentiate normally, a free surface seems to be imperative. Epidermal cells, in fulfilling their ultimate potentialities, move toward the surface, acquire keratohyaline granules, the granules dissolve, the cells fill with keratin, die, become greatly flattened and sometimes scaly, and eventually are sloughed off the epidermal surface. Exactly how much of this process is truly differential, and how much is the result of the cell's decreasing supply of nourishment in being pushed farther from the source thereof is not a subject for discussion at this point. An important fact seems to be that a free surface is needed to complete normal differentiation of epidermal cells. If this were not true, it would seem that in the tissue under consideration concentric bodies would not form about a specific point, but evidence of keratinization would be found throughout the epitheloid nests. Such a specific point in this case is that single epidermoid cell which appears first in the lumen of the youngest corpuscle. Whether this cell has undergone hypertrophy, degeneration, or whether it had already commenced differentiation before invagination

occurred, cannot be stated from a study of the tissue. Whatever be the cause, the result is evident: The cell undergoes a change in shape or orientation, with reference to its immediate neighbors, thus forming a small space between one or more surfaces of the cell and those adjoining. Once this space occurs in the epitheloid nest, differentiation progresses rapidly and uninterruptedly at successive concentric levels about the central cell, as the axis of the new corpuscle. Those cells immediately adjacent to the free area acquire keratohyaline granules, the granules dissolve, and the cells become squamous. A single lamella of keratinized cells is formed. A concentric space appears about the initial lamella, and additional lamellae are added from the surrounding epitheloid mass. The result of the activity is the establishment of concentric layers of keratinized cells, such a mass of cells being recognized as a concentric corpuscle.

Since the corpuscles herein described agree in detail with those produced in epidermoid carcinoma, it is probable that parallel phenomena establish the latter, and that the corpuscles described herein are homologous with epithelial pearls.

The important point under discussion, however, is a consideration of the possible significance of the concentric corpuscles in the interpretation of the corpuscles of Hassall. Romieu ('31) has already described epithelial pearls in the palatine tonsils, and has stated, with reference to these bodies, "*Par tous leurs caractères on peut les identifier à des corpuscules de Hassall.*" To the supporters of the theory of the epithelial origin of the corpuscles of Hassall there is, I believe, no cogent reason for not homologizing epithelial pearls with the thymic corpuscles of Hassall. Nevertheless, there still remains the question of the possible origin of Hassall's corpuscles from endothelial cell hypertrophy. Should the latter interpretation prove to be correct, the homology suggested by Romieu could no longer be sustained.

It is a singular fact that all efforts to arrive at a generally acceptable interpretation of the significance of Hassall's corpuscles by a study of the thymus have, to this date, yielded

conflicting opinions. Jordan and Horsley, as described earlier in this paper sought, therefore, an explanation in the study of certain abdominal lymph nodes.

The writer suggests that the tissues described in the present paper are especially favorable to a consideration of this problem, by virtue of the following characteristics:

1. The presence both of small, involuting blood vessels, and of concentric corpuscles in the implant site. Do any of the blood vessels present evidence of the formation therefrom of concentric corpuscles?

2. The absence of pathological conditions in the tissue. The corpuscles are not found in a carcinoma. An objection may be interposed at this point to the effect that the abnormal presence of the invaginated epidermis in the dorsal musculature constitutes per se a pathological condition. If, however, the concentric corpuscles found in the thymus are actually remnants of an epithelial anlage, then (as has been suggested by Reinke in a personal communication), their presence constitutes a pathological condition for the race, but a normal condition for the individual.

3. The fact that the essential conditions are fulfilled for drawing an analogy between the experimental tissue and the medulla of the thymus. There is present a network of undifferentiated mesenchyme which has migrated into the implant site. In this connective tissue stroma are found the cells of the blastema of the regenerating tissues, free white blood cells, and small ingrown blood vessels. Isolated in nests, or continuous with the surface epidermis, are masses of cells of epithelial origin foreign to the site, but which were carried into the site along with the implant. Thus, both the thymus, and the tissue in question contain a transposed epithelium, unconnected with its point of origin by a lumen (as in exocrine glands) and lacking evidence of potentiality for differentiation in another direction (which potentiality is possessed, for instance, by the thyroid, wherein the epithelial cells are specialized to produce colloid). The assumption of a broad analogy between the implant site and the thymus should be

as valid as the assumption that the spleen, or that abdominal lymph nodes, provide conditions analogous with the thymus. It is suggested that the latter cannot be referred to as a lymphoid body to a greater degree than it may be referred to as an epitheloid body. The analogy in the present instance is based not only on the general histological character of the structures involved, but also on their similarity of derivation.

4. The tissue in question, while presenting a broad analogy with the tissue of the thymus, has certain morphological features which may provide additional aids in the interpretation of the phenomenon under consideration. Perhaps the most important of these features is the fact that, whereas the epitheloid nests are surrounded by blood vessels, there has been no actual invasion of the nests themselves by blood vessels or by reticular cells. The integrity of the epitheloid nests has been maintained.

If the tissue under consideration be considered broadly analogous with the thymus then conditions, at least with respect to the involuting blood vessels and the epitheloid nests, should be sufficiently similar to permit parallel processes to occur in both. Equal opportunities seem to be provided, therefore, by the thymus and by the tissue under consideration for the formation of concentric corpuscles. From what origin, then, are the concentric corpuscles formed? The presence of concentric bodies is confined to the epitheloid nests.

Observation shows, however, the presence of involuting blood vessels in the vascular areas of the tissue, cross sections of which resemble concentric corpuscles. This superficial resemblance is due in part to the fact that the endothelial cell nucleus is often hypertrophied and, with the cytoplasm, occupies a crescentic position about the lumen of the vessel. The deeply staining collagenous fibers of the site are often densely applied in concentric layers at the periphery of the endothelium accentuating the resemblance. The bodies so constituted would have to be considered young corpuscles. The fact that no blood vessels have invaded the epitheloid nests, however, precludes the possibility of the origin of the con-

centric corpuscle from occluded blood vessels. Moreover, no red blood cells are found in the lumina of the youngest concentric copuseles, and no morphological series may be established wherein the blood vessels may be shown to evolve into concentric corpuscles.

Thus, in the tissue under consideration, the concentric corpuscles are exclusively epitheloid (epidermal, hence ectodermal) in origin. They have no genetic continuity with endothelial cells of blood vessels. Since there is no pathological condition manifest in the tissue, the presence of corpuscles seems to signify a normal, stratified squamous epithelial differentiation procedure, occurring in a limited space, and in the absence of a free surface.

In view of the analogous conditions shown to exist in the thymus, and in the implant site, the author is constrained to believe that the same phenomena are responsible for the formation of the thymic corpuscles of Hassall. The findings herein reported seem to emphasize the possibility of the derivation of these bodies from ectoderm as reported by Norris.

A serious objection to the drawing of an analogy between the implant sites and the thymus may be proposed in view of the fact that the epitheloid nests are compacted, and do not form an epitheloid cytoreticulum as is the case in the thymus. However, this condition of compacted epitheloid nests was achieved in the thymus experimentally by Jolly ('23) who, by Röntgen radiation, destroyed the lymphocytes in the thymus and observed not only that the epitheloid cells had become compacted as in the surface epithelium, but also that, in the absence of these non-epithelial elements, there was an actual increase in the number of Hassall's corpuscles. This seems to be further evidence that the epitheloid elements alone are responsible for the formation of the corpuscles.

In the tissues under discussion, the concentric corpuscles have no teleological significance, and cannot be considered centers of antitoxic or other physiological activity. Their concentricity is an expression of physical, not of physiological, conditions.

SUMMARY

1. Concentric corpuscles, produced experimentally in nests of epidermis mechanically invaginated in the dorsal musculature of *Triturus*, are described.

2. These corpuscles result from the keratinization of active epidermoid cells in the absence of a free surface.

3. A single epidermoid cell undergoes keratinization, a space appears between this cell and those adjoining, surrounding cells become oriented concentrically about the initial cell, and keratinization progresses centrifugally, producing concentric strands of squamous cells separated by concentric spaces.

4. A few keratinized cells are sloughed off the inner surface of the corpuscle, into the lumen.

5. These corpuscles are homologous with epithelial pearls.

6. The experimental tissue is analogous to the thymus with respect to the presence both of involuting blood vessels and of epitheloid tissue.

7. Concentric corpuscles are limited to the epitheloid nests in the tissue under consideration.

8. It is suggested that the same conditions obtain in the thymus, and that thymic corpuscles are derivatives solely of an epithelial anlage, possibly ectodermal in nature.

9. The concentric corpuscles described herein have no teleological significance, and are the result of physical, and not of physiological, conditions.

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PLATE 1

EXPLANATION OF FIGURES

Photomicrographs of sections through implant sites. Figures 1 to 4 are at a magnification of $\times 75$. Figures 5 to 8 are at a magnification of $\times 250$.

1 Epidermal tongue invaginated into implant site. Formative concentric corpuscle shown near tip of tongue.

2 Concentric corpuscle shown in epidermal tongue connected to thickened epidermis of wound by narrow isthmus of cells.

3 Nest of epidermoid cells carried into implant site by needle and isolated therein.

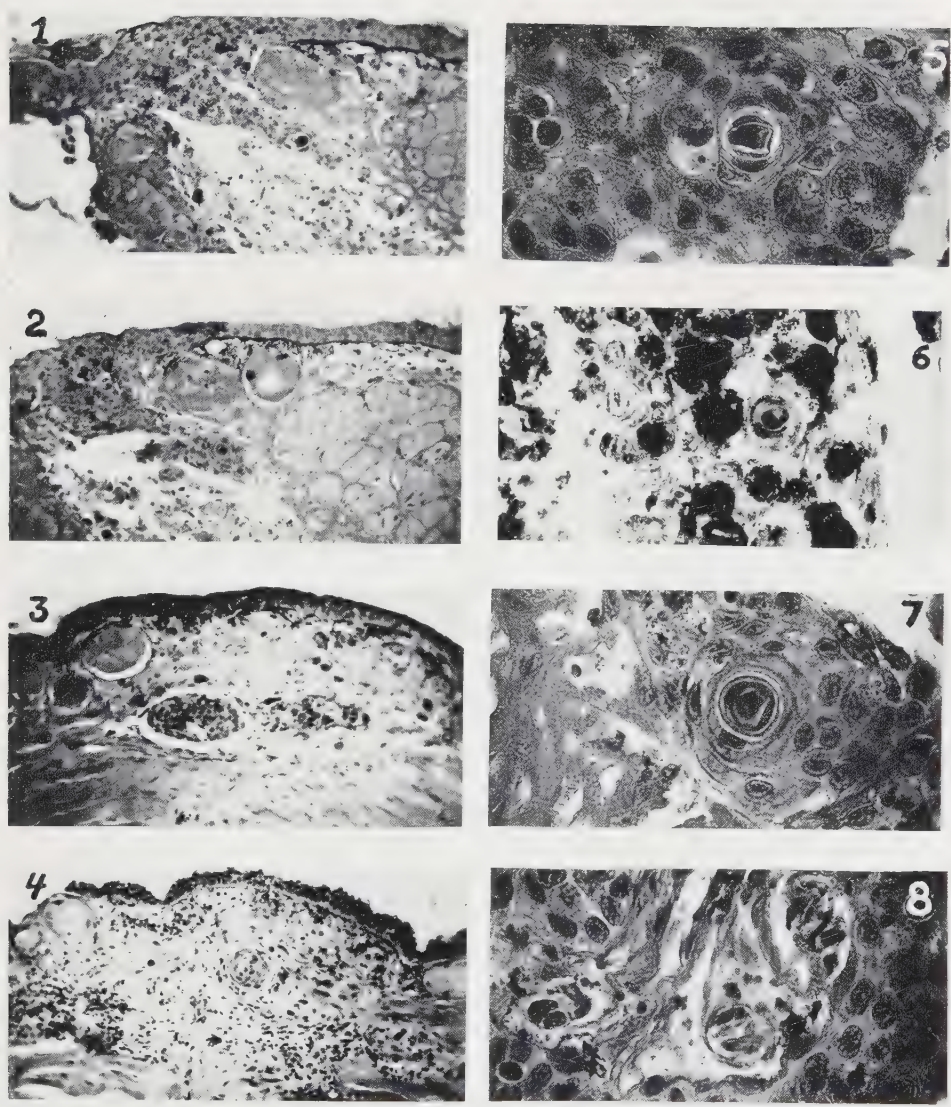
4 Nest of isolated epidermoid cells rounded up under pressure of surrounding tissue or surface tension. Entire nest appears keratinized but no concentric corpuscle is shown. Probably a true epithelial pearl.

5 Young concentric corpuscle in epidermoid nest. Note large, squamous cell in lumen.

6 Concentric corpuscle in epidermal tongue. Many melanophores present in site, introduced by needle.

7 Concentric corpuscle in epidermoid nest. Orientation of surrounding epidermoid cells indicates corpuscle still growing by accretion.

8 Blood vessels and mesenchyme in implant site.



AN X-RAY STUDY OF MALE PELVES ¹

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SIX TEXT FIGURES AND THREE PLATES (SIX FIGURES)

By means of Thoms' method of x-ray pelvimetry, we recently determined the size and shape of the pelvic inlet of 582 primigravid white women from the obstetrical clinic of the New Haven Hospital and of 104 nulliparous young white women from a somewhat different racial stock and a much more privileged economic group. Most of the latter were students of the Yale School of Nursing. The type of pelvis which is described in textbooks of anatomy and of obstetrics as the 'normal' female pelvis was found in only 14.9% of the clinic women and in only 5.7% of the student nurses. Indeed, only 35.2% of the clinic women and 13.5% of the student nurses had the type of pelvis which, according to the anthropological literature, is proper for white women (Greulich and Thoms, '39).

In an attempt to determine whether the pelvis of the present-day white male differs from its textbook counterpart as much as does that of the female, we have made antero-posterior and lateral roentgenograms of the pelves of sixty-nine Yale medical students. The purpose of this paper is to describe the types of pelves found in that group and to compare them with the male pelvis as it is described in textbooks of anatomy.

¹ Read before the American Association of Anatomists, Boston, Massachusetts, April 8, 1939.

² Aided by grants from the Clinical Research and Teaching Fund of the Yale University School of Medicine and from the General Education Board of the Rockefeller Foundation.

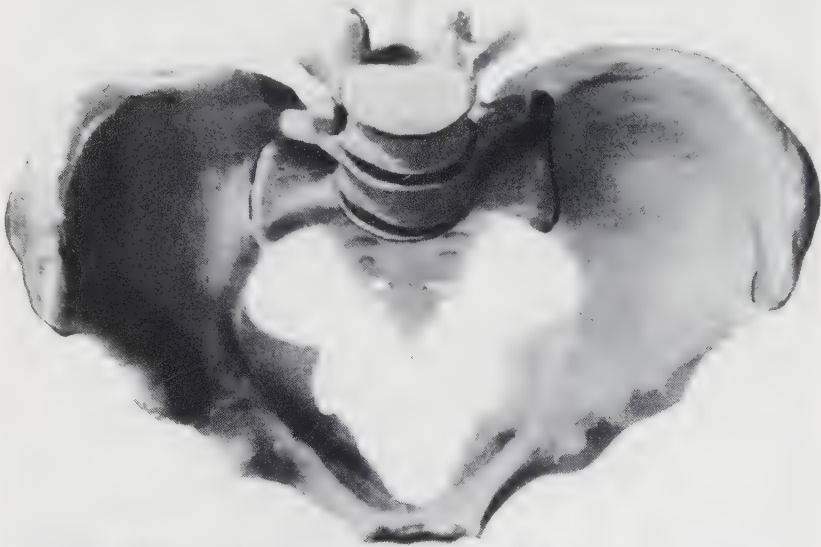
The superior aperture, or superior strait, of the true pelvis is said to be heart-shaped in the male and to have an antero-posterior diameter which is smaller than its maximum transverse diameter. The relative size of these two dimensions is most conveniently expressed as the pelvic index. This index is the antero-posterior, or conjugate, diameter of the pelvic inlet $\times 100$ divided by its maximum transverse diameter. By means of this index, it is possible to group together pelves which show the same degree of antero-posterior flattening or elongation, regardless of their absolute size. William Turner (1885) classified pelves into three types on the basis of their pelvic index: Those with an index of less than 90 he termed 'platypellic,' those with an index of from 90 to 94.9, 'mesatipellic,' and those with an index of 95 or more, 'dolichopellic.'

Of the sixty-nine men in our series, five, or 7.2%, were platypellic; ten, or 14.5%, were mesatipellic, and fifty-four, or 78.2%, were dolichopellic. Their pelvic indexes ranged from 77.0 to 121.0, the average index of the group being 100.5. This is considerably higher than the index of 80.8, which is the average of the indexes quoted by Martin ('28) as those reported by Turner, Verneau, Topinard, Flower and Krause in the nineteenth century. The pelvic indexes found by these investigators are still cited as typical of white males by authors of modern textbooks of anatomy. Only two of the sixty-nine men of our series had a pelvic index of less than 85 (77.0 and 84.8).

The illustration reproduced as figure 1 is taken from Spalteholz' *Hand Atlas of Human Anatomy*³ and is very similar to the male pelves which are pictured in other modern textbooks. Attention is directed especially to the shape of the superior aperture and to the fairly straight sides of its forepart.

³ Figure 1 is reproduced here with the kind permission of J. B. Lippincott Company, the publishers of the English editions of Spalteholz' *Hand Atlas of Human Anatomy*.

The pelvic inlet can be traced rather accurately from films made according to Thoms' method of x-ray pelvimetry. Such tracings were made of the pelvic inlet of all men studied and are shown in figures 2 to 6, in which they are grouped according to pelvic type. In each figure the individual tracings are arranged in the order of magnitude of their pelvic index, the tracing with the lowest index being on the left in the first horizontal row.



199. Männliches Becken, *pelvis*, von vorn oben

Fig. 1 Male pelvis pictured in Spalteholz' Hand Atlas of Human Anatomy.

It is evident from the tracings that the shape of the pelvic inlet of most of the men of our series is quite different from that described in the textbooks as typically masculine. Indeed, the pelvic inlet of some of our males so closely resemble that of some of the student nurses of our earlier series that it would be difficult in certain cases to determine the sex of the individual from the shape of the pelvic inlet alone.

The sixty-nine men in our series did their undergraduate work in thirty-five different colleges or universities in this country or abroad. Twenty-three states and one foreign country (England) were represented in the group. In view of this rather wide geographical distribution, it seems probable that our series is a more representative sample of present-day university men in this country than its small number would suggest.

For the most part, the men studied were well developed physically and belonged to a relatively privileged economic group. It is interesting to note that they, like the similarly

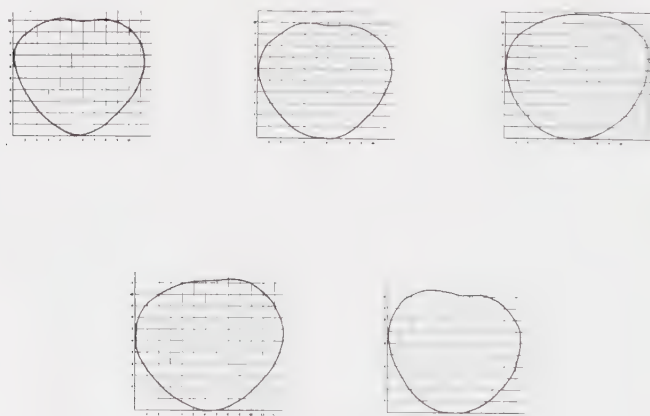


Fig. 2 Tracings of platypellie male pelvises.

avored student nurses of our previous series, show a high incidence of dolichopellie pelvises, 73% of the student nurses and 78.2% of the men having that type of pelvis.

The average pelvic indexes of the men of our present series and of the women whom we studied previously are considerably higher than those reported by anatomists and anthropologists 50 years ago. It appears, therefore, that the shape of the pelvic inlet in both sexes has changed materially during the past half century. This, of course, presupposes that the bulk of the population at the time those early de-

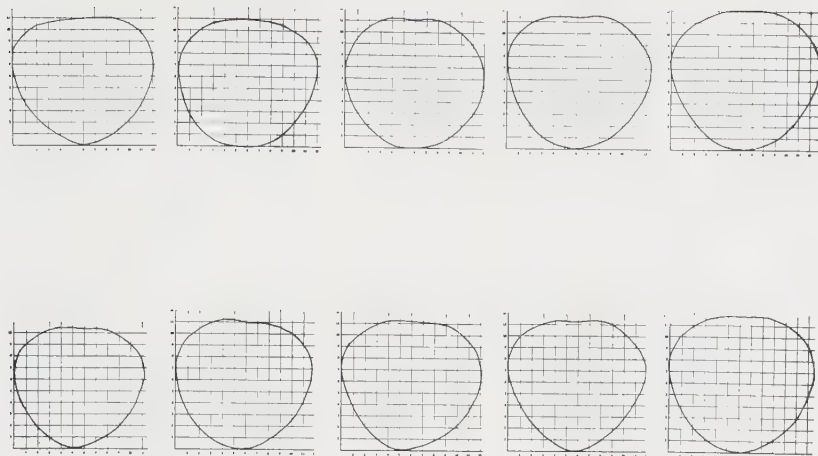


Fig. 3 Tracings of mesatipellie male pelves.

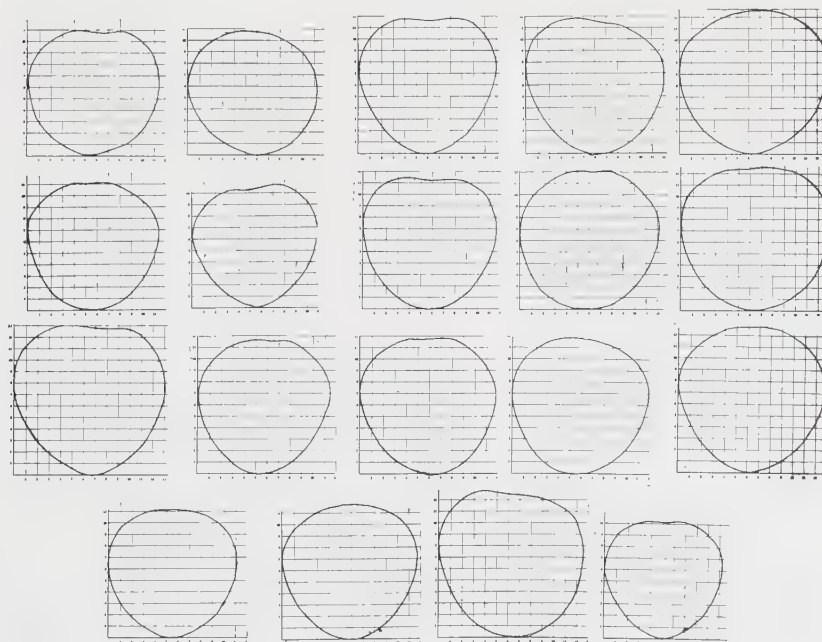


Fig. 4 Tracings of dolichopellie male pelves.

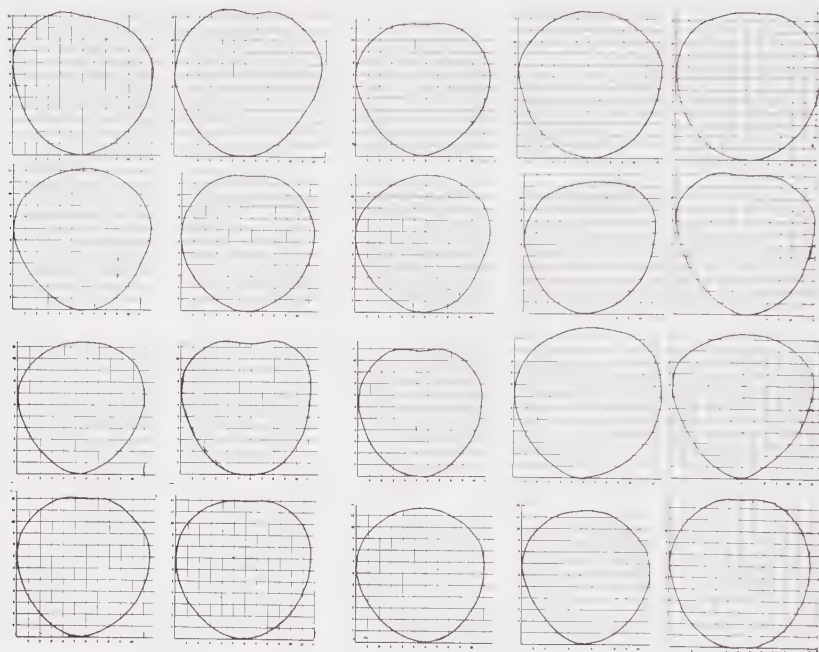


Fig. 5 Tracings of dolichopellic male pelves.

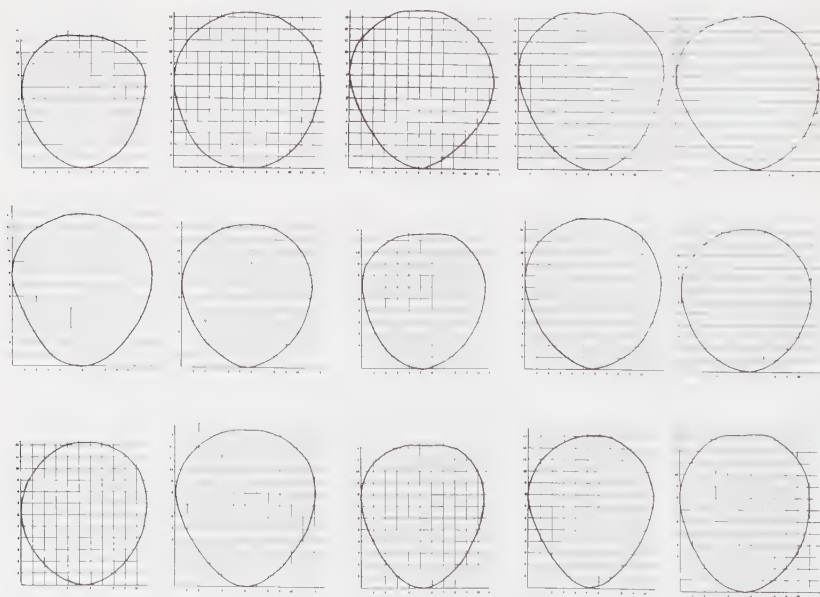


Fig. 6 Tracings of dolichopellic male pelves.

scriptions were made had the same kinds of pelves that their less fortunate contemporaries brought with them to the dissecting room, since those early descriptions were based almost entirely upon skeletal material from that source. The possibility that they were somewhat different should not, however, be forgotten. In this connection, it is well to remember that, in general, dissecting room subjects are from the least privileged class of the population from which they were derived. It follows that they may be expected to show, to an especially marked degree, the results of the nutritional and other inadequacies which so often accompany extreme poverty. One must be cautious in generalizing from observations made on dissecting room material.

Our findings indicate that the pelvic inlet of the male is as variable in shape as is that of the female; for although the males of our series were predominantly dolichopellic, there were also some mesatipellic and some platypellic individuals among them. It is evident, therefore, that there is, in our population at least, no one type of male pelvis, just as there is no one type of female pelvis.

The average posterior sagittal diameter of the inlet of the male pelves was only slightly smaller than that observed in a recent series of 200 primiparous white women from the obstetrical clinic of the New Haven Hospital which has not previously been reported. Thus, the plane of the maximum transverse diameter of the inlet of our males was situated only slightly nearer the sacral promontory than was the case in those females.

In the great majority of the men the forepart of the pelvic inlet was more rounded and its pubic portion somewhat wider than is suggested by the usual description of the male pelvis. The designation 'heart-shaped' or 'kartenherzförmig' is, therefore, rather inappropriate for the pelvic inlet of the males of our series, because in the great majority of them the maximum transverse diameter was not displaced far enough dorsally, nor was the forepart sufficiently narrow to fit that description.

An extra pelvic roentogenogram was made of some of our men in order to determine the character of the pubic angle. It was found to be quite similar to that described as masculine in the textbooks. In most of the males the ischial spines were distinctly heavier than those of the females and they projected farther into the pelvic cavity. There were, however, a few cases in which the spines were not so prominent, in men who were apparently quite as masculine as the others. The greater sciatic notches of our males, though in some cases somewhat wider than they are usually described, were definitely narrower than those of the females of our earlier series.

Through the kindness of Dr. J. B. Hamilton, of the Department of Anatomy, we were able to study the pelves of two hypogonadal brothers, aged 23 and 26 years, respectively, whom he had under observation. Photographs of antero-posterior and lateral roentgenograms of their pelves and photographs of their external genitalia are reproduced in plates 1 to 3. The shape of the superior aperture of both pelves is practically identical with that of many of the apparently normal males of our series. Asymmetries in the shape of the inlet comparable to those observed in these two cases were rather common in our series, though their similarity in these two brothers may have a familial basis. Some features of their pelves are especially instructive.

The greater sciatic notches of the older brother's pelvis, which is shown in figure 10 are definitely wider than those observed in the great majority of the males of our series and in that respect they resemble slightly those of the normal female. The ischial spines (fig. 12) are, however, definitely male-like in size and in the degree to which they intrude into the pelvic cavity. The external genitalia of that subject (fig. 8), though markedly hypoplastic, are better developed than are those of his brother. The ischial spines of the younger brother (fig. 11) are not so prominent as those of the older one and this might perhaps be regarded as an indication of a tendency

toward femaleness; but the greater sciatic notches (fig. 9) fit exactly the textbook descriptions of these structures in the normal male. It is noteworthy that, in this case, typical male notches are associated with the extremely hypoplastic genitalia shown in the illustration (fig. 7). The penis is so small that it scarcely merits that designation; and it is only with the aid of a good reading glass that the outlines of the diminutive testes can be distinguished in the photograph.

SUMMARY AND CONCLUSIONS

The shape and dimensions of the pelvic inlet of sixty-nine white adult male students were determined by means of x-ray pelvimetry and the findings compared with the description of the male pelvis given in modern textbooks.

1. Their pelvic indexes were found to range from 77.0 to 121.0, the average index being 100.5. This is considerably higher than 80.8, the average pelvic index reported by anatomists and anthropologists of the nineteenth century and still cited as typical of white males in current textbooks. It appears, therefore, that the shape of the pelvic inlet has changed materially since those early descriptions were made.

2. The superior aperture of the true male pelvis was found to be as variable in shape as is that of the female, dolichopellic, mesatipellic, and platypellic types being represented in our series with a frequency of 78.2, 14.5 and 7.2% respectively.

3. In the great majority of the male pelves studied the forepart of the inlet was more rounded and its pubic portion somewhat wider than is suggested by the usual description of the male pelvis.

4. The average posterior sagittal diameter of the inlet of these males was found to be only slightly smaller than that observed in a recent series of 200 primiparous white women. This indicates that the plane of the maximum transverse diameter of the inlet of the males was situated only slightly nearer to the sacral promontory than was the case in those females.

5. The pubic angle of the males in this series in whom it was determined resembled very closely that described as masculine in the textbooks.

6. In most of the males the ischial spines were distinctly heavier than those of the female and they projected farther into the pelvic cavity. There were, however, a few cases in which the spines were not so prominent, in men who were apparently quite as masculine as the others.

7. The greater sciatic notches of these males, although in some cases somewhat wider than they are usually described, were definitely narrower than those of the females of our earlier series.

8. Pelvic roentgenograms of two adult hypogonadal males disclosed in both a pelvic inlet indistinguishable in size and shape from that of many apparently normal men. In one of these hypogonadal males the ischial spines were typically masculine, as were the greater sciatic notches of the other subject. The association of these supposedly masculine pelvic characters with extremely hypoplastic genitalia in these two cases demonstrates that those characters may occur even in the absence of normal testes. They are, therefore, not necessarily a reliable indication of the degree of maleness or femaleness of the individual possessing them.

We wish to acknowledge our indebtedness to Dr. Hugh M. Wilson, Associate Professor of Radiology in the Yale University School of Medicine, who generously made available the facilities of his department for this investigation, and to Mr. Laurence Cowles, Technician in Radiology, who made the roentgenograms.

We feel particularly grateful to the medical students who cooperated in the study, especially to those first-year men who participated despite their obvious misgivings concerning possible untoward effects of x-ray pelvimetry on the male.

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PLATE 1

EXPLANATION OF FIGURES

7 and 8 The external genitalia of the two hypogonadal brothers whose pelvic roentgenograms are shown in figures 9 to 12. Note the extremely small genitalia and the sparse growth of pubic hair. (Figs. 7, 9, and 11 are of the younger brother; figs. 8, 10, and 12 are of the older brother.)



PLATE 2

EXPLANATION OF FIGURES

9 and 10 Lateral pelvic roentgenograms of the two brothers whose external genitalia are shown in figures 7 and 8. The greater sciatic notches in figure 9 are very similar to those usually described as masculine. The notches shown in figure 10 are somewhat wider than those usually found in normal males, but they are not wide enough to be considered typically feminine.

The sacrum of the male is said to be more concave from above downward than is that of the female. In these brothers, however, the narrower, more 'male-like,' sciatic notches shown in figure 9 are associated with a sacrum which is much straighter than that of the pelvis shown in figure 10, which has much wider and, therefore, less 'male-like' greater sciatic notches.

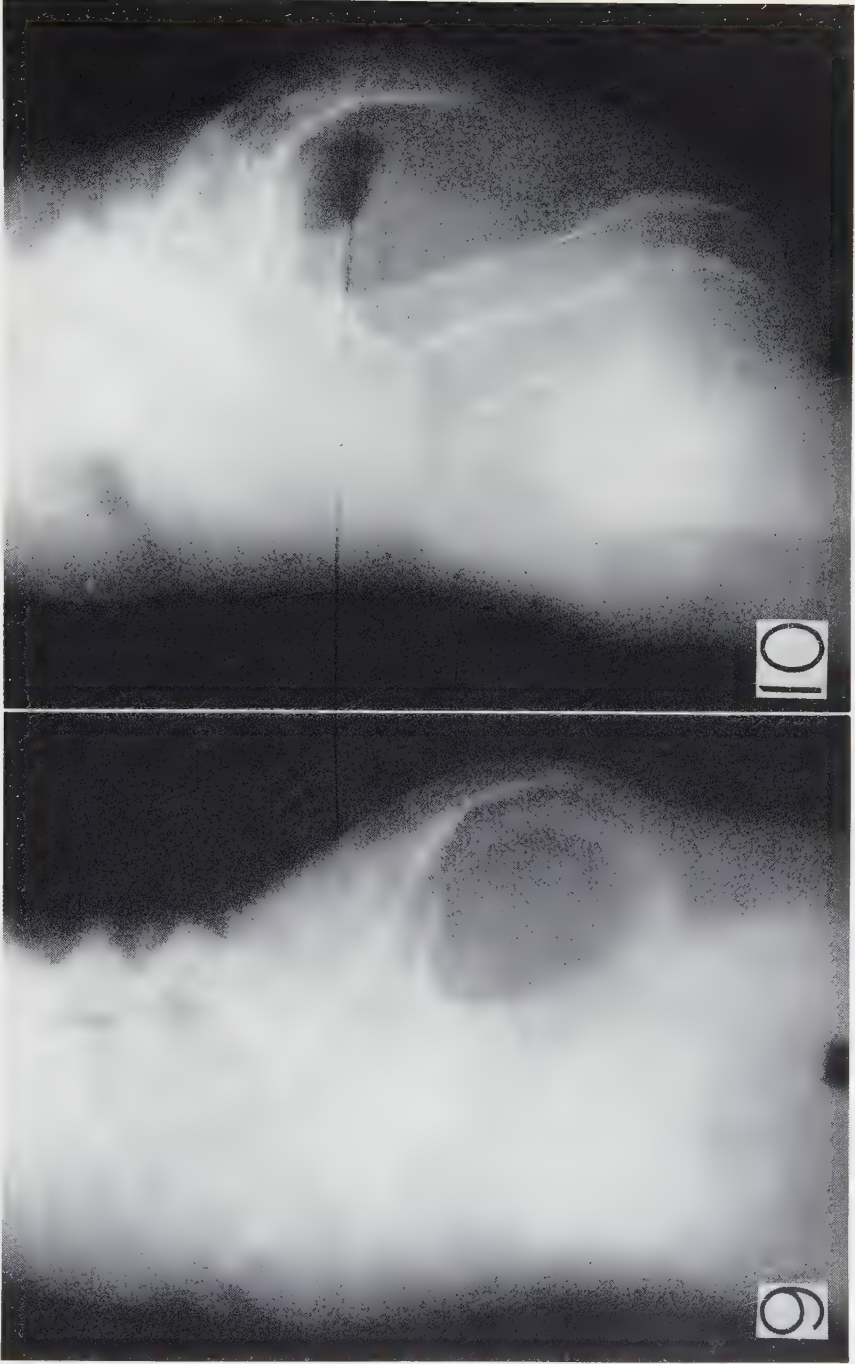
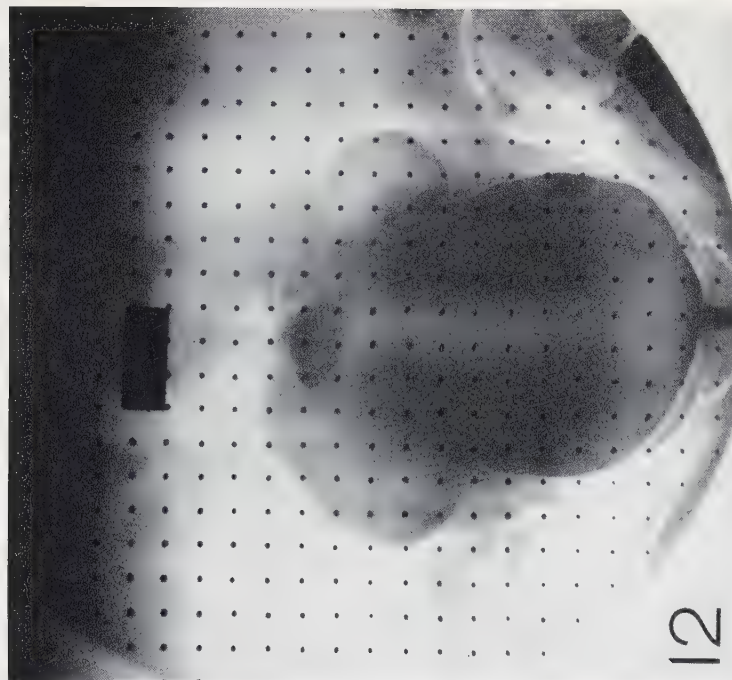
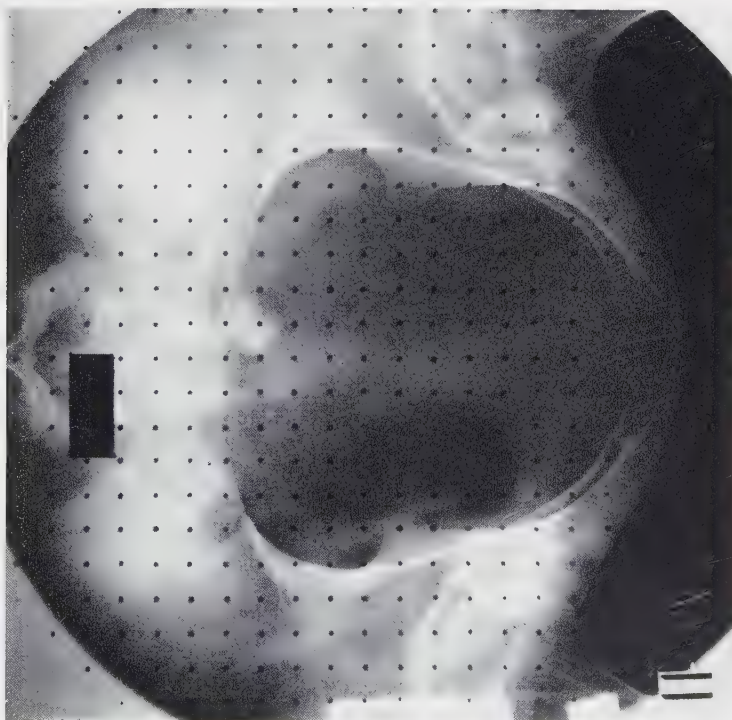


PLATE 3

EXPLANATION OF FIGURES

11 and 12 Antero-posterior pelvic roentgenograms showing the superior aperture and the ischial spines of the two brothers.

Note that the heavier, more 'male-like,' ischial spines in figure 12 are associated with the relatively wide greater sciatic notches shown in figure 10. Conversely, the narrower, 'male-like' notches in figure 9 are associated with the rather poorly developed spines seen in figure 11.



INFLUENCE OF THE NERVOUS SYSTEM ON BONE AND JOINTS

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That functional impairment of the central or peripheral nervous system by pathological or traumatic processes often results in marked involvement of the bones and joints is a well-established clinical observation. Nevertheless, it is still a moot question as to whether such skeletal changes result from the loss of specific trophic nerves to the bony structures, or occur secondary to disuse, infection, loss of sensation, or to muscle atrophy.

Profound degenerative changes occur in skeletal muscle following interruption of its nerve supply, and it has therefore been quite reasonably concluded that the motor nerves exert a definite trophic influence over striated muscle (Tower, '39). However, in the case of bone innervation, one is dealing with a quite different phenomenon. Muscle deprived of its nerve supply is incapable of functioning in a normal manner. Numerous studies involving bone innervation and denervation, on the other hand, seem to indicate that were it possible to isolate the skeleton from the nervous system, without simultaneously denervating surrounding tissues, especially muscle, no alterations, neither of a functional nor anatomical nature, would appear. Notwithstanding this technical difficulty, it will be seen in the following report that it is possible for the bones and joints of a limb deprived of its sensory and autonomic innervation to maintain a perfectly normal anatomical state, and probably to function normally, over a period of years.

¹ The major part of the experimental procedures reported here were completed while the authors were members of the Department of Anatomy, Stanford University.

A variety of experiments have been executed in an attempt to determine the effect of denervation on bone growth and structure. Certain workers have sectioned peripheral nerves, subsequently studying the bones of the denervated limb, but, because such procedures usually resulted in muscle paralysis, and consequent disuse of the limb distal to the nerve section, and because of the frequency of infections, often resulting in osteomyelitis and purulent joints, most of the early results must be heavily discounted. Schiff (1851), Milne-Edwards (1860), Nasse (1880), Kapsammer (1898) and Ghillini (1898), reported bone changes following peripheral nerve section, whereas Friedle and Schinz ('25) and Sulger ('25) report no skeletal changes.

During war abundant human material is usually available in which the results of peripheral nerve injury on bone may be studied. Fleischhauer ('15), Riedel ('16), Lehmann ('17) and Maliwa ('17), have all reported atrophy after bullet wounds involving peripheral nerves.

Grey and Carr ('15) found no bone alterations in one dog, 2½ months after sectioning dorsal roots supplying the skeletal parts being studied, but there was a marked atrophy after cutting either ventral roots or peripheral nerves. Similarly Eloesser ('17), found no changes in cat ribs deprived of their posterior root innervation. He reported no difference in chemical composition, no atrophy nor weakening of deafferented limb bones, although the joints of such cats often showed traumatic arthritic-like changes.

Growth of skeleton deprived of its thoracolumbar autonomic nerve supply has been shown to occur quite normally in young experimental animals (Takahashi, '22; Cannon and co-workers, '29; Bacq, '30, and Bisgard, '33). Key and Moore ('33) and McMaster and Roome ('34) report that sympathectomy causes no noticeable deviation from the usual in duration or course of fracture repair. However, Harris ('30) and Harris and McDonald ('36) claim that lumbar sympathectomy accelerates longitudinal growth in the paralyzed limbs of children who have suffered poliomyelitis.

Apparently no nerve fibers penetrate hyaline cartilage (Stohr, '28). Sensory endings, principally of the Pacinian type, were found in the periosteum (Miskolczy, '26, and Sfameni, '00), and in the joint capsules (Tello, '22; Oda, '35, and Leriche, '30). Myelinated and unmyelinated nerves, with free and lamellated endings have been described as being in the bone marrow of young animals (Rossi, '32). Small medullated fibers have been found entering the Haversian canals and the medullary spaces, usually associated with blood vessels, and therefore thought to be vasomotor in function (Stohr, '28). Although de Castro ('25, '30) reported nerve twigs ending on osteoblasts in growing bone, he found no nerves in fully formed bone. Recently, Hurrell ('37) reported silver impregnation of nerve fibers in the bone matrix of an adult cat femur in one instance. His inability to obtain similar results in a large number of attempts is attributed to technical difficulties. He suggests that there are "sensory and effector endings required for a reflex control of bone growth and maintenance."

There is no evidence that any fibers arising in the motor cells of the spinal cord terminate in bone. Furthermore, integrity of the motor nerves to the striated muscle is essential in a study of this nature in order to prevent muscle atrophy and the attendant secondary skeletal atrophy. Section of the posterior roots of the lumbo-sacral spinal cord, plus removal of the lumbar sympathetic chain, should completely isolate the skeletal parts of the lower extremity from the central nervous system. If the intactness of the sensory or of the thoracolumbar autonomic pathways is necessary for the maintenance of normal function and structure of bone, such procedures should be followed by observable physiological or morphological skeletal alteration. Obviously, interruption of these pathways also deprives other tissues of the limb of their sensory and sympathetic innervation, and therefore modifies to a certain extent the normal environment of the bones and joints. As mentioned before, it would appear physically impossible to isolate the skeletal parts alone, without

interfering with their blood supply or muscle attachments. However, it is felt that the effect of sympathectomy and deafferentation of a limb on its skeletal parts may be interpreted satisfactorily, especially with negative findings, in the face of this compromise.

METHODS

Fifteen adult cats were used in these experiments. In eight, the lumbar sympathetic chain (L1 or 2 through L7 or S1) was removed unilaterally, in seven bilaterally. In thirteen of these animals, the 4th, 5th, 6th and 7th lumbar, and the 1st, 2nd and 3rd sacral dorsal roots were sectioned unilaterally, in four cats before the sympathectomy, in nine afterward. The interval between the operations varied from 3 weeks to 1 year. Thus in thirteen of the above animals one hind limb was deprived of both its sympathetic and sensory innervation. In two cats, the lumbar sympathetic chain was removed without deafferentation. The interval between sympathectomy and autopsy varied from 125 to 1445 days, that between deafferentation and autopsy, from 14 to 1394 days. A tabulated summary of the experiments follows.

Table of experiments

Cat no.	Date sympathectomized		Date dorsal roots sectioned. L4, 5, 6 and 7, S1, 2 and 3.	Termination of experiment	No. of days between sympathectomy and experiment termination	No. of days between dorsal root section and experiment termination
	Left	Right				
342		10-26-32		7-17-34	617	
520		8-24-32		7-19-34	695	
216		6-16-32	5-16-31	7-18-34	763	1159
236		7-22-31	7-23-32	7-24-34	1098	732
238		7-23-31	7-22-32	7-20-34	1093	724
276		9-24-32	10-21-31	7-27-34	672	1010
277		7-22-32	10-22-31	7-26-34	735	1008
288		7-19-32	12-29-31	7-21-34	733	934
411	7-31-34	7-31-34	9-20-34	7-15-38	1445	1394
419	8-20-34	8-20-34	9-18-34	3- 1-36	549	520
420	8-28-34	8-28-34	9-22-34	5- 1-36	641	616
421	8-28-34	8-28-34	9-26-34	9-11-35	378	350
422	12-24-34	12-24-34	4-26-35	5-16-35	144	21
424	12-26-34	12-26-34	4-20-35	3-25-38	1185	1070
426	12-28-34	12-28-34	4-18-35	5- 2-35	125	14

All operative procedures were carried out under ether anesthesia. Careful inspection of the denervated limbs was constantly made in order to take the necessary measures to prevent the formation of traumatic ulcers. The entire series of animals was carried to autopsy without the occurrence of ulcers or infection. Nine of the cats were permitted the freedom of a large outdoor cage. Six were kept at all times in standard animal cages, $1\frac{1}{2}$ by $1\frac{1}{2}$ by 2 feet.

Roentgenograms were made of the complete lower extremities of ten cats before autopsy. Following fixation, further roentgenograms were made of the pelvis and lower extremity. Excellent fixation for the preparation of the microscopical material was obtained by the intra-arterial injection of 10% formalin. Representative sections of all articular surfaces and shafts of both the normal and denervated pelvises and lower extremities were decalcified in 5% aqueous nitric acid, embedded in celloidin, and sectioned at 15μ . In four cases, the femurs, patellas, tibias and fibulas were cleaned of their soft parts, oven dried, treated with several changes of ether to remove the fat, and redried to a constant weight at 110°C .

RESULTS

The animals in whom unilateral sympathectomy alone was performed showed no roentgenological, macroscopic, microscopic or gravimetric differences between the bones or joints of the operated and normal limbs. These cats appeared to use their sympathectomized limbs normally in all respects. As previously mentioned, experimental work has demonstrated that the presence of the thoracolumbar autonomic nerve supply is not necessary for the normal growth of bone. Similarly, our observations indicate that no functional or anatomical alterations appear in adult bone deprived of its sympathetic innervation for as long as 3 years. Recent work completed in the laboratory of one of the authors (J.C.H.) shows that there may be regeneration of preganglionic thoracolumbar fibers to lumbar and sacral ganglia remaining after lumbar sympathectomy. We do not believe, how-

ever, that such regeneration would be of sufficient amount to negate our conclusions, because in most instances the second sacral chain ganglion was the only one left in situ. In the absence of postganglionic neurons, function does not seem to be resumed.

In considering the results of deafferentation, the matter of trauma to the anesthetic joints becomes very significant. With the single exception noted below, those animals which were kept isolated in small cat cages during the postdeafferentation period (over 3 years in two cats) showed no alterations from the normal in any of the bones and joints of the anesthetic limb. The denervated bones of two animals in this group were dried to a constant weight following ether extraction of the fat. No significant differences were found in the weight of the individual bones of the anesthetic hind limbs as compared with the normally innervated ones. Nor could careful microscopic examination of representative portions of the articular surfaces and shafts of the denervated bones reveal any alteration from the normal. The x-ray studies of these deafferented hind limbs were also completely negative as regards detectable changes from the normal.

The desensitized right hind limb bones and joints of cat 424, deafferented 1070 and sympathectomized 1185 days previously to autopsy, and kept throughout this period in a small standard cage, showed no alterations except in the talo-cuboidal joint. The cartilage of this joint was eroded away with underlying bony sclerosis, a change similar to that seen in the arthritic hip joints to be described later. Although this animal did not use the deafferented limb to any great extent, the anesthetic foot showed plantar flexion, and the cat rested on the dorsum of the foot, thus overflexing the talo-cuboidal joint. No other alterations were found in the skeleton of the anesthetic limb in this cat.

Those cats permitted the freedom of a large outdoor animal cage used their anesthetic hind limbs considerably. The deafferented limb was usually carried extended at the knee and hip, the dorsum of foot dragging along the ground. In this

position, the hip joint was hyperextended and consequently areas of its articular surface unaccustomed to weight bearing were constantly being traumatized. This condition led to erosion of the articular cartilage with underlying bony sclerosis. Since these changes have been fully described elsewhere (Corbin, '37), we shall omit a detailed microscopic description in this paper. It is sufficient to say that the alterations were characteristic of a deforming arthritis and were obviously the result of attrition in an anesthetic joint. Probably because of the underlying bony sclerosis of the cancellous bone, the femurs exhibiting this arthritic change were slightly heavier than the same bones of the normally innervated limbs. In no case has atrophy or weight loss been present in femurs whose heads showed arthritic changes, nor has any detectable microscopic change been found in the shafts of such bones. The other joints of these limbs appeared entirely normal. It is important to stress therefore, that the only change found in the deafferented limb bones of animals which used the anesthetic leg considerably was an attrition arthritis of the hip joint, with a slight increase in weight of the corresponding femur. Three of the animals in this group of ten were deafferented for periods of about 3 years.

DISCUSSION

Our experiments indicate that adult bone and joints maintain their normal structure and function when isolated from the thoracolumbar autonomic system for long periods of time.

In these experiments, deafferentation has never resulted in bone atrophy. The slight increase in weight of those deafferented femurs which showed marked deforming alterations of the head, is apparently the result of the same stimulus which caused the new bone formation in the medullary spaces beneath the eroded cartilage. Since these changes occurred only in that group which exercised their deafferented limb considerably, it would seem that such alterations are the result of constant trauma in an anesthetic joint. Although a great

deal has been written concerning the protective role played by the pain mechanism, too little attention has been given to the similar protection afforded by the proprioceptive mechanism. Endings, receptive to this type of sensibility, are numerous in the region of the joint capsule, and, to a certain extent, are found in the periosteum. Deafferentation thus eliminates the protection furnished the joints by these proprioceptors, and motion beyond the normal range follows. This form of sensibility, however, cannot be termed trophic.

Bone is a living tissue, and extraordinarily sensitive to its functional requirements. Hence, any experiments modifying its accustomed function are apt to result in changes in its structure. We have eliminated muscle paralysis, infection, and to a certain extent, disuse, but have not been able to preserve normal function completely. We have, therefore, found alterations where they would be expected, that is, in the main weight bearing joint of the anesthetic limb, but only when that joint was used in an abnormal fashion. Here, portions of the articular surface, unadapted for weight bearing, are worn away, and there appears concomitantly a compensating bony sclerosis affecting the spongy bone of the femoral head. The loss of the joint sensibility prevents the maintenance of the normal range of motion, and consequently the articular surface is severely traumatized. The bones and joints of those animals whose movements were limited by confinement in a small cage showed no alterations.

Our experiments, however, offer no evidence that bone nutrition or structure is dependent in any degree on its nerve supply, *per se*, but only secondarily when interruption of the innervation of surrounding tissues alters its normal function.

SUMMARY

In thirteen cats the skeletal parts of one hind limb were completely denervated for periods of from 2 weeks to 3½ years following lumbar sympathectomy and section of L4-S3 dorsal roots. In each of two cats the lumbar sympathetic chain was removed unilaterally without deafferentation. Roentgeno-

logic, gravimetric, macroscopic and microscopic studies were then made of the denervated and normally innervated bones and joints.

No changes were seen in bones and joints completely denervated (deafferented and sympathectomized) for as long as 3 years in those animals whose movements were restricted by confinement in standard small animal cages. In cats which ran free in a large outdoor cage, constant trauma of the anesthetic hip joint, used considerably and in an abnormal fashion, resulted in an attrition arthritis with concomitant sclerosis of the subarticular cancellous bone. The other denervated bones and joints in the latter animals showed no alterations from the normal.

Because the structure of the skeletal parts may be maintained in a normal condition for over 3 years following complete denervation, we conclude that bone and joints are not supplied with nerves having a specific trophic function. Deafferentation results in a loss of the protection afforded by the proprioceptive mechanism and, therefore, may lead to attrition arthritides in those joints subject to constant trauma.

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THE FUNCTIONAL SIGNIFICANCE OF THE CAPILLARY BED IN THE BRAIN OF THE OPOSSUM

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SIX FIGURES

The correlation that is usually made of relative vascularity to relative intensity of metabolism in various regions of the brain has been questioned in recent discussions, both as to its justification and value. A close examination reveals in fact that little is known about the functional significance of the capillary bed of the brain. The assumption that more capillaries per unit of volume mean higher metabolism is reasonable, and it has been generally accepted in view of the striking parallelism existing between the cytoarchitecture and the angioarchitecture of the brain, which could hardly be explained on any other basis. The assumption, however, has not been proved directly. Physiological methods thus far available cannot be applied for a determination of the metabolic rate of single nuclei in the brain.

An attempt to enter the field is made here with a study of the brain of the opossum, the unusual vascular pattern of which has been described by Wislocki and Campbell ('37). These investigators have made the following interesting suggestion:

In spite of being true end-systems, adjacent units overlap intricately and profusely by interdigitation of their respective capillary loops. Indeed, the hairpin-like terminal capillaries of several neighboring systems share the territory between

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them about equally, thereby nourishing the brain tissue from independent sources. Because of the marked overlapping, occlusion of a vascular unit in the opossum's brain might not lead to complete infarction of the territory supplied by it.

This statement would mean that, whereas in the brains of all other mammals thus far studied the blood vessels are of the network type and behave physiologically like end-arteries, in the brain of the opossum the anatomical end-arteries do not have the same physiological significance. The theory is an interesting one which calls for a study of the effect of experimental occlusion of the end-arteries in the brain of this animal.

The observations made on experimental emboli in the brain of the opossum not only demonstrated that the blood vessels are end-arteries in a true physiological sense, but indicated also a way of determining the area of supply of a single capillary. This determination, although possible thus far only in a favorable territory, namely in the cerebellum, is of importance with respect to the problem of the relationship between regional differences in vascularity and metabolic rate. Finally, the results obtained in the present work offer an opportunity for discussion of the question as to whether capillaries in the brain may open and close as is the case in other organs.

MATERIAL AND METHODS

In nine adult opossums, *Didelphys virginiana*, a sterile suspension of lycopodium spores in saline solution was injected into one of the carotid arteries. The immediate effect varied with the amount injected, and the animals suffered from the multiple emboli produced in the respective side of the head in different ways which it is unnecessary to describe in connection with the problem here presented. After various intervals ranging from 8½ hours to 49 days following operation, the brains were removed in deep nembutal anesthesia and fixed in Zenker-formol or in 95% alcohol. They were embedded in nitrocellulose and cut serially. After alcohol fixa-

tion, the sections were stained with toluidin blue or thionine (Nissl preparation); and after fixation with Zenker-formol, with iron hematoxylin and a modified Masson's stain (Foot, '33). The observations here described were made mainly on one animal which had been allowed to live for 48 hours after the injection and in which an exceptionally high number of small lesions had occurred.

There were furthermore available a number of opossum brains fixed in various ways by perfusion and immersion, mostly embedded in nitrocellulose and stained after Nissl or with the Masson trichrome stain. Besides single sections or shorter series of sections from brains injected with carmine-gelatine or India ink-gelatine, a complete series was made from the olfactory bulbs to the medulla of a brain that had been injected with carmine-gelatine according to the technique suggested by Bensley ('38). Every alternating section was cut 20 μ and stained with toluidin blue, and was mounted with the corresponding unstained 200 μ section.

1. Anatomical and physiological end-arteries

The fact that the capillary bed is in free anastomosis throughout the mammalian brain and the brain vessels are, therefore, not end-arteries in a strictly anatomical interpretation, proved to be of no importance concerning the pathogenesis of vascular lesions in the brain. Although the arteries of the brains of man and most mammals are not end-arteries in an anatomical sense, they nevertheless react functionally as such, i.e. they are physiological end-arteries. As a matter of fact, the blood vessels in the brain behave like end-arteries in the case of sudden occlusion, simply because the anastomoses described by Goldstein ('14), Pfeifer ('28, '30), etc. are not numerous enough and the nervous tissue is too sensitive in regard to lack of oxygen and undergoes disintegration before an efficient collateral circulation can be established by new anastomosing vessels (Wolff, '38; Sunderland, '38). Even if the occlusion is gradual, as for instance in the case of endangiitis obliterans (Sträussler, Friedmann and Schein-

ker, '38), the foci correspond in shape and extent to the area of supply of the occluded vessel. The situation, therefore, is that the brains of man and most other mammals, with the exception of the opossum and the kangaroo (Craigie, '38 a) and probably the marsupials in general, are vascularized by blood vessels that are end-arteries, not in an anatomical but in a physiological sense. An entirely different situation seems

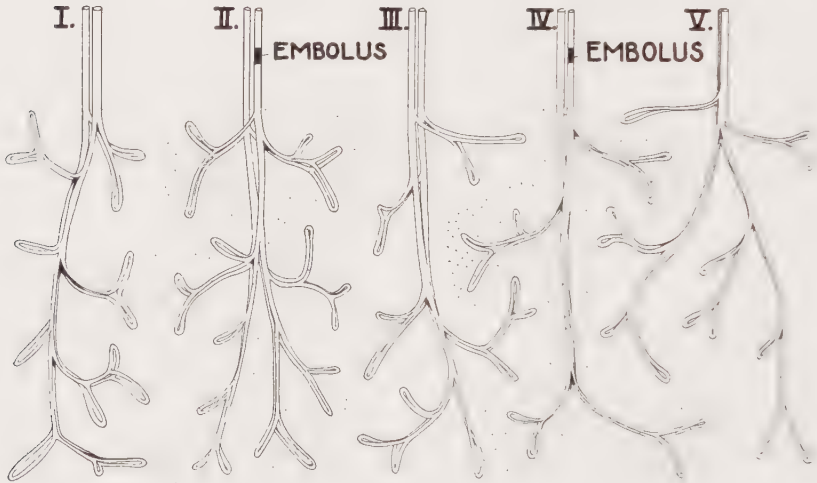


Fig.1 Schematic drawing of end-arteries of opossum's brain. The capillary loops of II do not interdigitate with those of I and III. An embolus in II would result in an ischemic lesion as indicated by stippled area. Such lesions do occur. As a rule, however, the capillary loops overlap each other as in III, IV and V. If III and V could supply the territory of IV when the latter is occluded, IV would be an anatomical, but not a physiological end-artery. Actually the embolus in IV causes an ischemic lesion corresponding in shape to that of the occluded vessel, indicated by stippled area. Lesion is shown in photomicrograph, figure 2.

to exist in the brain of the opossum. As quoted above, Wislocki and Campbell suggested the possibility that complete infarction might not occur in the case of occlusion of a single end-artery in the brain of this animal, on account of the interdigitating arrangement of the capillary loops belonging to different neighboring vessels (fig. 1). This question was studied by means of experimentally produced embolisms.

The occlusion of a blood vessel in the brain of the opossum by a lycopodium spore results in the disintegration of nerve cells in essentially the same way as it does in other mammals. A histopathological description of the foci is, therefore, unnecessary. Since the interest here centers on the functional value of the end-arteries, the chief concern is with the shape and extent of the lesions resulting from emboli in capillaries and precapillary vessels. It is seen that the occlusion of a cerebral artery leads to the complete disintegration of the nervous elements supplied by the capillary branches of the artery. The shapes of the lesions in the opossum's brain correspond to the ramifications typical of the blood vessels in the brain. In the cortex and the brain stem the result is, however, not so strikingly clear as it is in the cerebellum, where the densely packed cells of the granular layer offer a better background and reveal a sharp demarcation of the territories affected. The cerebellum provides, so to speak, a fine-grain picture of what is happening in all parts of the brain, while the coarser grain of the larger and more scattered nerve cells does not present so vivid a demonstration, as is shown in figures 2 and 3.

From these observations it can be seen that the anatomical end-arteries of the opossum's brain also behave functionally as such, that is, the occlusion of a blood vessel by an embolus causes ischemic disintegration of all the nerve cells supplied by this vessel. The overlapping of the interdigitating capillary loops is not sufficient to insure the supply of the territory normally vascularized by the occluded vessel.

Another feature of minor significance that may be mentioned is the straight-lined boundary which at times is observed between an ischemic and a normal territory. It is in contrast to the pictures of branched areas as described above, and suggests that there are areas of vascular supply without an overlapping of capillary loops (compare fig. 1). Patterns such as these are found in sections of normal brains injected with carmine-gelatine, but it can hardly be asserted on the



Fig. 2 Ischemic lesion caused by occlusion of blood vessel in granular layer of cerebellum of opossum, corresponding to the branches of vessel. (For comparison with injected preparation see Wislocki and Campbell, '37, fig. 3, p. 184; or Scharrer, '38, fig. 6, p. 408.) Fix. alc. 95%, nitrocellulose, 20 μ , thionin. $\times 100$.

basis of a study made of the normal brain that there are no loops entering from a neighboring vessel. The findings in brains showing embolic lesions suggest that in the cerebellum both arrangements exist, namely areas largely supplied by one vessel only, where occlusion of the vessel results in a more

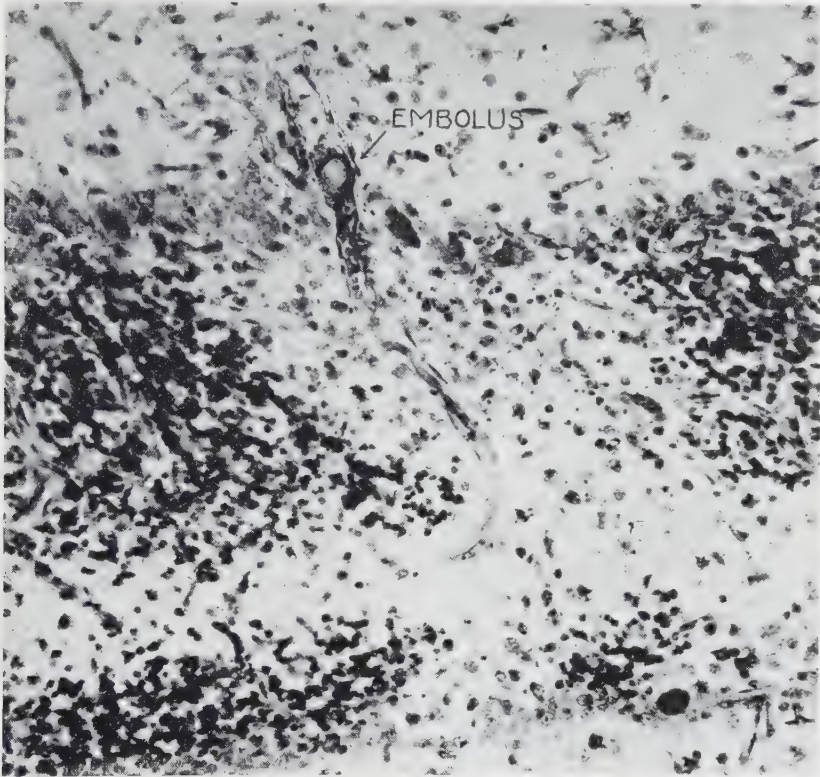


Fig. 3 Occlusion of a capillary in cerebellum of opossum by an embolus (lycoperidium spore). Fix. alc. 95%, nitrocellulose, 20 μ , thionin. $\times 200$.

or less closed lesion (fig. 1, II); and districts supplied from different sources, where the afflicted area corresponds to the branches of the occluded vessel (fig. 1, IV). The first case seems to be rare, while the second one represents the rule.

*2. The significance of quantitative regional differences
in the capillary bed*

When Wislocki and Campbell ('37) announced the discovery of the vascular pattern of the opossum's brain it was of some interest to compare the angioarchitecture of a brain with so peculiar a vascular pattern as that of the opossum with the angioarchitecture of other mammals showing the familiar network type. It was readily demonstrated that in the case of the opossum the angioarchitecture is as highly differentiated as is the cytoarchitecture, i.e. the density of the capillary supply corresponds closely to the density of cells in different nuclei. (Compare Scharrer, '38, p. 404, fig. 3, a, b; or Gerard, '38, p. 330, fig. 135, A, B.)

In the same way as in the rat (Craigie, '38), in the opossum the capillary bed is richer in the cortex than it is in the white matter, and richer in the nuclei of the brain stem than in the fasciculi. In the cerebellum the capillary bed of the granular layer is richer than that of the molecular layer, whereas the white matter is the least vascularized. There is little point in presenting detailed descriptions of the observations here, since careful quantitative determinations have been made by Craigie for the rat. The significance of quantitative data has been contested by some workers who argue that, as in muscle, factors such as blood flow, utilization of oxygen by the tissues, the opening and closing of capillaries, etc. play a more important role for the adjustment of the blood supply to meet the needs of different brain centers than does the capillary bed. Another difficulty is seen (Gerard, '38) in the discrepancy between the results of the determination of the metabolic rate of peripheral nerve as contrasted with that of the cortex, in a comparison with the capillary beds of both structures. Whereas the capillary beds are in the ratio of 1:2, the metabolic ratio is 1:30. A later discussion will indicate how far such discrepancies can be used effectively in an argument against the value of the anatomical findings.

At the beginning of an investigation of the significance of regional vascular differences in the brain it seems useful to study the mutual relationships into which nerve cells and capillaries can enter in this region. It has been pointed out in a previous paper (Scharrer, '37) that thus far four possible relationships between nerve cells and blood vessels have been observed.

1. Nerve cells and blood vessels show no immediate relationship except differences in vascularization in different areas.

2. Nerve cells follow the blood vessels and keep in contact with the vascular walls as far as possible. The best example of this type is the optic ganglion of the cephalopods.

3. The blood vessels are determined by the nerve cells, as in the case of the pericellular capillaries of some hypothalamic nuclei and the dentate nucleus in mammals.

4. The most intimate relationship between blood vessels and nerve cells exists when capillaries appear as endocellular blood vessels, as for instance in the supramedullary nerve cells of certain fishes.

In the brain of the opossum the vast majority of the nerve cells shows no definite relation to the blood vessels, and the latter in turn follow their own pattern independently all over the brain. The second type, namely that of the dependence of nerve cells upon blood vessels, is found in the supraoptic nucleus. As in the optic ganglion of the squid, groups of nerve cells follow the blood vessels in a manner similar to that known for some vertebrates (W. C. Meyer, '35). The third and fourth possibilities are also found in the supraoptic and paraventricular nuclei, insofar as pericellular capillary loops are directly attached to single cells. The significance of the particular cell—blood vessel relationship in the supraoptic and paraventricular nuclei will be discussed in another paper in connection with the secretory activity of these nuclei.

With the exception of the few cases just mentioned, it is permissible to conclude that in the brain of the opossum

neither the pattern of the capillary bed nor that of the nerve cells is determined by direct cell—blood vessel relationships. The nerve cells are arranged in functional units (cortical layers, nuclei) and so are their processes (fiber bundles, white matter, neuropile). The blood vessels in their turn are arranged in the described pattern of capillary loops. The basic principle of the loop pattern does not depend upon the nerve cells. It is independent of the living nervous tissue, since it has been shown that the capillaries may grow as loops even into dead brain tissue (Scharrer, '39). But the number of capillary loops per unit of volume of brain tissue apparently depends upon the density of the nervous elements. This fact is clearly demonstrated by the already mentioned parallelism of the angioarchitecture and the cytoarchitecture (p. 326). The question as to whether the differences in the capillary beds of various localities in the brain indicate metabolic differences has been answered thus far in the affirmative, because it could hardly be conceived that so elaborate an angioarchitecture should have no functional significance. Recently, however, doubt has been cast upon the validity of the argument, and the value of quantitative studies in the field of brain vascularization has been generally questioned.

The problem under discussion here is, therefore: Are the distances between the capillaries of functional significance, and should they be considered as indications of the metabolic rate of the tissues lying between the capillaries? An answer to this question is attempted by the diagrammatic illustrations of figure 4. The capillary loops C and D in the figure are supposed to be at such distance from each other that the nerve cells lying between them are adequately supplied. It is not known, however, whether the radii of supply of both capillaries overlap, or whether their ends coincide with the midline between the two capillaries. The question might be decided by taking out and studying a single capillary. This procedure was accomplished in the case of capillary B in figure 4. If the diffusion radius of A and C respectively is greater than half of the distance between A and B and also

between B and C, there should result no cellular disintegration between A and C, or only a very narrow strip of disintegration. If, however, the diffusion area of each capillary has its boundaries midway between the neighboring capillaries, it is to be expected that the total territory of B would undergo ischemia whenever B is occluded. The width of the ischemic area should then be of the same order as the full distance between two capillaries, i.e. equal to twice the efficient diffusion radius of the occluded capillary.

With this question in mind, a study of the material obtained from experimentally produced emboli and injected

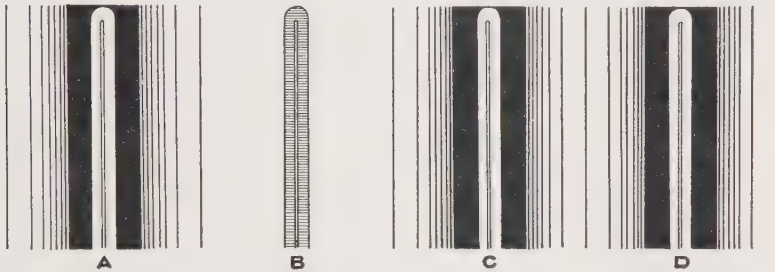


Fig. 4 A, B, C, D, four capillary loops. The diffusion radius of each is indicated by lines. All nerve cells in the territory between C and D are adequately supplied. Capillary B is occluded by an embolus and no blood circulates in its lumen (indicated by hatches). For survival of the cells in area between A and C, the diffusion radii of these two capillaries should meet in B, and should, therefore, normally overlap that of B. This is not the case; the cells disintegrate around the occluded vessel in a circle, with a radius of half the distance between two capillaries (fig. 2).

brains shows that the latter expectation proves actually to be true. Since in the granular layer of the cerebellum preserved and disintegrated nervous tissues are well separated by sharp boundaries, the diffusion radius of a capillary can be estimated directly. It appears that it is equal to one-half of the distance from one capillary to the next. The distance can be computed from the pictures of the lesions in comparison with the intercapillary distances as obtained from injected specimens (fig. 5). The result is that the diameter of the narrow strips of ischemic territory as pictured in figure 2, which re-

sult obviously from the occlusion of a single long capillary loop, is equal to the distance between two capillary loops. In the granular layer of the cerebellum this distance with little variation is found to be $50\ \mu$ in sections of fixed and embedded material. The diameter of the narrowest ischemic strips also is $50\ \mu$ in material treated in a similar way. A capillary, therefore, can maintain the life of the nerve cells only in its immediate surroundings, in a radius of about one-half of the

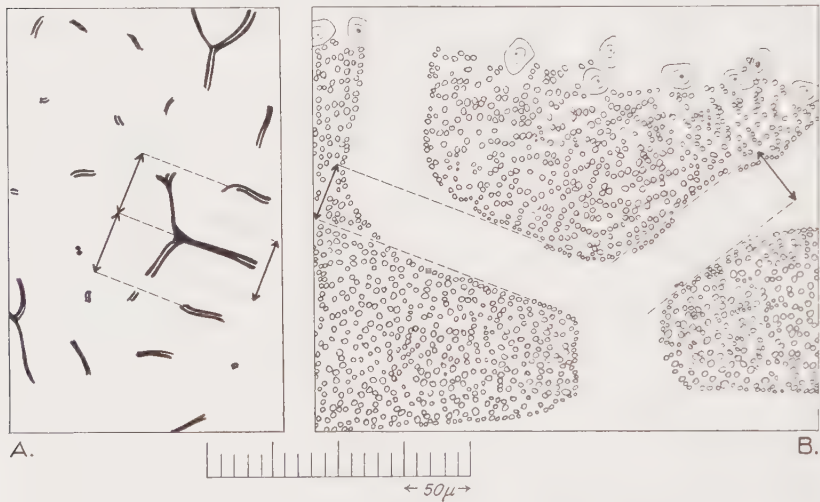


Fig. 5 A. In a $20\ \mu$ section of the injected granular layer of the cerebellum of the opossum the distance between two capillaries is about $50\ \mu$. B. Part of the photomicrograph of figure 2 is redrawn at the same magnification as A. The diameter of the ischemic strip is also $50\ \mu$. The diffusion radius of a capillary is, therefore, half the distance between two capillaries, as indicated in A.

distance between two capillaries. If one capillary fails to function, the neighboring capillaries cannot prevent the death of the nerve cells ordinarily supplied by this blood vessel, as the supplies of blood are used up by the nerve cells of their own territories before they can reach the cells of the afflicted area. The distance between two capillaries in the granular layer of the cerebellum of the opossum is, therefore, directly related to the metabolic rate of the cells supplied by these

vessels, Since the use of a double supply of blood from different sources is obviated, a regular, economic, capillary pattern results.

A regular pattern of equidistant capillaries is possible in the case of loops of the type found in the brain of the opossum. In a network pattern the blood in the venous ends of each capillary stretch is partially exhausted, and distances between capillaries probably must vary in a way so as to insure an adequate supply to the nerve cells, i.e. the distance between two or more arterial ends of capillaries can be greater than that between the venous ends. Since, however, the blood may flow in an opposite direction in neighboring capillaries, the resulting capillary pattern is more irregular in a network than in an end-arterial system, and an equidistant capillary pattern can, therefore, not be expected in a network system. There is no need for a variation of distances in the opossum and there is consequently less irregularity in the distances between the capillaries, since the problem of the deterioration of the blood in the venous end of the capillary is solved by the close association of the two members of a capillary loop. Craigie ('38 a) recently discussed in a highly interesting way the functional meaning of this arrangement. As a matter of fact, the association between the two blood vessels in the brain of the opossum is so intimate that each pair may be regarded as one vessel (fig. 6), as far as its relationship to the surrounding tissue is concerned. Each pair of blood vessels is sheathed by a pia-glia membrane common to both capillaries and is embedded in the same space of Held's glia chambers. This close association of arterial and venous limb obtains in all cerebral blood vessels of the opossum's brain, immediately from their entrance in the nervous tissue on the surface of the brain to the last capillary branches. Each capillary represents a hairpin-like loop, the two limbs of which run parallel to each other in close association, as indicated in figure 1. Craigie compares this type of capillary with the arrangement of the maternal and foetal vessels in the placenta of the rabbit, as described by Mossman ('26).

Craigie, with whom the writer is in full agreement and to whose point of view there is nothing to add, continues:

It seems possible that a somewhat analogous situation exists in the central nervous system which is vascularized by capillary loops. A spongy reticulum permits a more or less free distribution of blood in all directions through the tissues, but when the supply is entirely by closed, non-anastomosing loops, the venous limb of each loop would tend to carry less oxygen

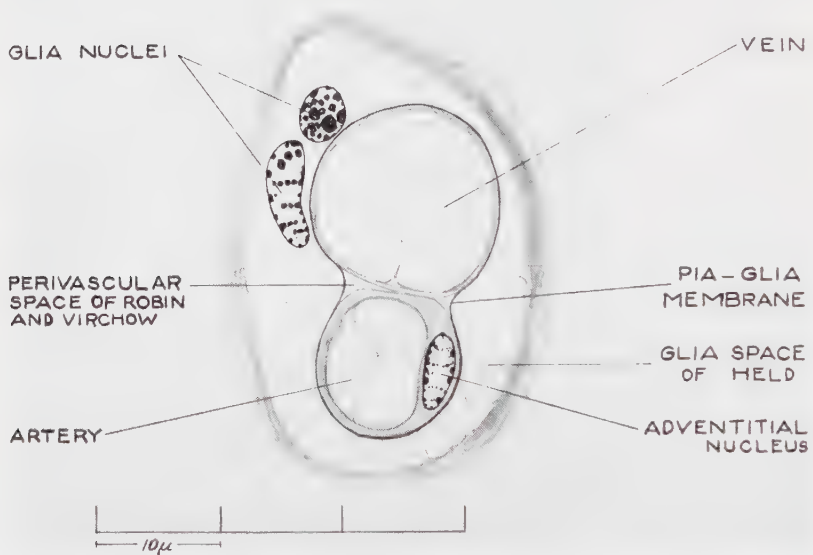


Fig. 6 Cross section through a pair of precapillary blood vessels in the opossum's brain. Zenker-formol, nitrocellulose, 7 μ . Mallory azan.

and nutriment and more waste products than the arterial limb. If the limbs were spread widely apart, the tissue immediately surrounding each venous limb would thus be supplied with blood poorer than that supplying the tissue surrounding each arterial limb. The two limbs being so closely associated as they are, however, the blood flowing along the venous limb and tending to decrease in purity is associated with blood of increasingly arterial character in the arterial limb. Thus a

relatively uniform condition of the blood throughout the capillary loop may be maintained and every part of the tissue through which the loop passes is more likely to have an adequate supply.

Summarizing, it may be stated that in the granular layer of the cerebellum the distance between two capillaries is equal to the width of the ischemic territory that appears when one capillary loop alone has been made ineffective by an embolus. Neighboring capillaries, therefore, share the territory between them half and half, and their diffusion area is limited by the requirements of the cells which they supply. In the case of the granular layer of the cerebellum, the capillaries could not lie further apart without producing a condition of inadequate care for strips of cells in the midline between two capillaries. If the capillaries were nearer to each other, it would do no harm to the nerve cells, but the vascular system would take up more space than is absolutely necessary. This would be possible only at the expense of the nervous tissue, for which no excess space is available inside the cranial cavity. But a system of capillaries distributed in regular distances well adjusted to the radius within which oxygen and nutriment brought by the blood are used up by the nerve cells, fulfills the main requirements of the cerebral vascularization, i.e. efficiency and economy. There may be and certainly are effective, in addition to the variations in the capillary bed, other regulatory mechanisms that allow a local increase or decrease of vascular supply by widening or narrowing the brain vessels, by changing the rate of blood flow, etc. But these questions are beyond the scope of the present paper, and with respect to this point the reader is referred to the articles by Wolff, Gerard, etc. quoted above.

DISCUSSION

The regional differences in the capillary bed of the brain are so obvious as to lead easily to the assumption that they represent regions having different rates of metabolism. This point of view has recently been contested (Gerard, '38, p. 331:

“(Clearly anatomical findings may be used only as the most primitive indication of metabolic differences”), because there seems to exist a discrepancy in that the capillarity of the cortex is a little more than twice as rich as that of the trigeminal nerve, whereas the ratio of oxygen consumption is 30:1.

It does not appear that this is an argument against the significance of the anatomical findings. Rather it seems to prove that it is not justifiable to make a direct comparison of vascularity and the metabolism of peripheral nerve on the one hand, and of the brain on the other. In the opossum the difference between the vascularization of the brain and peripheral nerve is indicated by the difference in the vascular patterns. In this animal, peripheral nerves, including the true cranial nerves, are vascularized by capillary networks, as are all the other organs, whereas the end-arterial loops are restricted to the brain and the optic nerve which is a part of the brain. A comparison between the capillary bed and the metabolism of peripheral nerve and of the cortex of the opossum is inadmissible, because their vascularizations differ in principle. But even where obvious differences do not exist, the discrepancy between metabolism and vascularization of peripheral nerve and of the cortex has no bearing upon the correlations obtaining between cortex, nuclei, and fiber tracts of the brain. If, however, the cortex and the white matter of the brain are compared, the results agree very well. Wolff ('38, p. 54) states:

Thus, as mentioned above, the gray matter of the human cerebral cortex has about 1100 millimeters of capillary per cubic millimeter of brain tissue, whereas the white matter approximates 300 millimeters, and seldom more than half the amount of the gray. This is comparable to the oxygen consumption in the cortex as compared to the white. The rabbit cortex takes up about 1.2 cu.mm. per milligram per hour of oxygen (Holmes, '32), whereas white matter takes up about 0.2 to 0.3 cu.mm. per milligram per hour, an amount well under half the consumption of the cortex.

Thus the differences in the basic metabolic rates of the various functional units of the brain are taken care of by fixed differences in the capillary bed. Increased activity of single centers is connected with a local increase of blood flow on the basis of the existing capillary bed. But this fact does not, of course, render meaningless the differences in the capillary bed, since functional adjustments of the capillary bed could not go so far in the brain as they go, for instance, in muscle, where capillaries can be opened and closed.

A mechanism of this type has in fact been discussed by Putnam and Alexander ('38, p. 551) with regard to the brain: "It appears probable that capillaries may function intermittently or in rotation in the nervous system as in other organs. Doubtless, therefore, isolated capillaries here and there may close without producing parenchymal degeneration." Talbott, Wolff and Cobb ('29) cut the cervical sympathetic nerve of the cat on the one side and observed an increase in the capillary bed of the homolateral cerebral hemisphere by measuring the capillary length in homologous areas on both sides. Expressed in this way, an increase in the capillary bed means the opening of additional capillaries consequent to the transection of the sympathetic nerve. An opening of capillaries as here indicated would also explain the observation of Craigie ('30) that the result is the filling of an increased number of capillaries during injection, if the pressure with which blood vessels are injected is raised.

Until data concerning the point are available for other mammals, the theory will be considered as applying only to the opossum. In the brain of this animal the capillary loops are distributed in different densities in various locations, but appear to be rather equidistant in a given locality. In order to reduce the capillary bed in a certain territory during inactivity, the number of capillary loops should be closed down in a way such as that which Krogh ('29) has shown obtains in the case of muscle. The situation thus created would correspond to an occlusion of the capillaries in question. It has been shown, however, that the occlusion of even a single capil-

lary causes damage to the nerve cells ordinarily supplied by that capillary, and that the vascular pattern seems to be a very economical one, which does not permit the functional inactivity of even a single capillary.

Without entering into a lengthy discussion of the problem before additional information comes from corresponding studies made on other mammals with different vascular patterns, it may nevertheless be suggested that the great difference existing between brain and muscle in regard to 'work' and 'rest' should not be overlooked. Whereas nerve cells are extremely sensitive to a lack of oxygen, there is far less difference in the metabolism of the working and the resting brain than there is in the metabolism of the working and the resting muscle. Certainly the difference is not so great that blood vessels are closed during the 'rest' period of the brain and opened during 'work.' It must be assumed that the deepest 'rest' of the brain takes place during hibernation. It seems incredible that nerve cells can be cut off from the blood supply for several months, even if their metabolism is considerably decreased. The brain needs a steady supply of blood, rather than a flexible supply such as muscle requires.

As far as mammals with a capillary bed of the network type are concerned, there also seems to be no indication of a physiological opening and closing of the capillaries, as Lennox ('36) has shown in man. Forbes and Cobb ('38) also arrive at the conclusion that the capillaries in the pia are always open and that the blood flow is remarkably steady. Corresponding results were obtained with long continued observations of the pial circulation by means of windows installed in the skull of rabbits (Wentsler, '36; Clark and Wentsler, '38). Physiological studies in man led Lennox, Gibbs and Gibbs ('38) to the conclusion (p. 293): "Under normal conditions, the circulation in the brain is probably more constant and steady than the circulation in the arm or leg. Unlike the muscles (except those of the heart), the brain is always active; its functional needs are always nearly maximal and the capillaries are always open."

Corresponding results were obtained in experiments made on the brain of the opossum. There also the cerebral capillaries must always be open to insure a steady supply of blood to the nervous tissues. It was shown that the distances between the capillary loops in a given region—the granular layer of the cerebellum—coincide with the efficient diffusion radius required for the supply of the cells. This finding justifies the linking of capillarity with metabolic rate and is in agreement with the results of Campbell ('39), who has shown that the oxidase content of different brain centers corresponds to their vascularization, and of Dunning and Wolff ('37) who have furnished evidence (p. 449) that: "nervous tissue involved in the transmission of the nerve impulse from neuron to neuron is more richly supplied with blood than nervous tissue involved in conduction only within the neuron."

It appears, therefore, that it is not advisable to draw conclusions in regard to the utilization of oxygen or the opening and closing of capillaries or other problems of brain vascularization from observations made on the skin, muscles, etc. This statement applies also to experiments concerning the effects of emboli, etc. made on the tongue of the frog, the rabbit's mesentery, etc., which since the days of Cohnheim (1872) are still freely substituted for studies made on the brain itself. In the case of the brain only the most general conclusions can be drawn from experiments made in other regions with respect to problems such as the role of thrombi and the vascular supply of ischemic territories. Any statement concerning the brain made on the basis of observations on other organs is of doubtful value.

SUMMARY

1. The blood vessels of the opossum's brain are not only anatomically, but also functionally true end-arteries. The embolic lesions resulting from injections of lycopodium spores into the carotid artery indicate that there are no areas nourished from different sources by the overlapping of capillary loops so as to prevent complete ischemia. The ischemic terri-

tory corresponds in shape and extent to the area of supply of an occluded vessel.

2. There are not more capillaries than are necessary to insure the supply of blood to the nervous tissue. The diffusion sphere of each capillary seems to overlap little or not at all that of the neighboring capillaries. The nearly equidistant distribution of the capillaries in each vascular area of the opossum's brain is to be viewed as the anatomical expression of the vascular economy.

3. There is no evidence to show that quantitative regional differences in the capillary bed of the brain should not be considered as correlated to differences in metabolism. The width of an ischemic territory in the granular layer of the opossum's cerebellum, resulting from the occlusion of a single capillary loop, corresponds exactly to the distance between two capillaries in the same region, as measured in injected specimens. The distance between two capillaries in the cerebellum indicates, therefore, the metabolic rate of the nerve cells which the capillaries supply.

4. It is very improbable that in the opossum's brain capillaries are closed and opened according to changing requirements during rest and activity in a manner similar to that known for muscle. The prompt ischemic reaction of the nervous tissue in cases of the occlusion of blood vessels by emboli makes it appear unlikely that under normal conditions any capillary in the brain of the opossum is ever closed for any length of time.

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REMARKS ON THE LYMPHATICS OF THE REPRODUCTIVE TRACT OF THE FEMALE RHESUS MONKEY (*MACACA MULATTA*)

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NINE TEXT FIGURES AND ONE PLATE (FIVE FIGURES)

Lymphatics of the female reproductive tract have been investigated extensively in man although interest has been directed mainly to the collecting trunks and regional drainage. The score or more of papers pertaining to the drainage of the different parts of the external and internal genitalia in man are reviewed by Jossifow ('30) and Rouvière ('32) and will not be presented here.

The question of the pattern and origin of the intrinsic lymphatic plexuses within the various parts of the mammalian reproductive tract has, on the contrary, received relatively little attention.

The ovaries. The intrinsic, injected lymphatics of the human ovary have been illustrated and described in some detail by Polano ('03). The injected lymphatics of the cow's ovary are well shown in three drawings presented by His (1865); Buckel (1874), on the contrary, in rabbits' ovaries, produced extravasation which he confused with lymphatics. Polano ('03) succeeded in demonstrating distended lymphatics in the ovaries of two dogs, by tying the collecting trunks near the hilus. Miss Anderson ('26) has given a complete description, accompanied by excellent drawings, of the lymphatics in the sow's ovary utilizing the method of injection and taking into account the changes accompanying the oestrous cycle.

The fallopian tubes. An account, accompanied by figures of the injected lymphatics of the tubes in man, has been given by Kroemer ('04). Miss Anderson ('27) describes in detail the injected lymphatics of the fallopian tubes of the sow, referring, as in her study of the ovary, to the changes accompanying the reproductive cycle.

The uterus. Intrinsic lymphatic plexuses in the human uterus have been described by Leopold (1874) and Kroemer ('06) in injected preparations, and by von Franqué ('06), who utilized the proliferation of cancer cells as a means of detecting the lymphatics. In two gravid human uteri Schick ('06) also demonstrated by means of injection the presence of lymphatics. In animals, the uterine lymphatics have been injected by Leopold (1874) in sheep, pig and rabbit, and by Mierzejewski (1879) in cow, mare, sow, sheep, dog and guinea pig. They have also been investigated by means of silver impregnation by Hoggan and Hoggan (1883), in a great variety of mammals.

The vagina. The lymphatics of the vagina appear to have received very little attention. Cateula ('30, '31) has briefly described the pathways of the collecting trunks in injected human material. Poirier (1889), from specimens injected with mercury, gives brief descriptions of two intrinsic lymphatic plexuses in the human, one in the mucosa, the other in the musculature. His observations concern, however, mostly the collecting lymphatics and the illustrations are highly schematized. Bruhns (1898) investigated the lymphatic collecting trunks in the vagina, in a series of injected human specimens, but in addition noticed that the lymphatics arise in a fine meshed intrinsic network lying immediately beneath the vaginal epithelium.

The sexual skin. The only mention that we find of lymphatics in the sexual skin of monkeys is the report of Aykroyd and Zuckerman ('38) that lymphatic trunks extending from the sex skin to the groin were outlined upon injecting trypan red intravitaly into the sex skin of one male and several juvenile female rhesus monkeys.

These investigations establish principally that lymphatics are present in abundance in the female reproductive tract. With the exception of the two modern studies of the ovary and fallopian tube of the sow carried out by Miss Anderson, they leave much to be desired in the way of description and portrayal of the intrinsic lymphatic plexuses. The figures accompanying the older articles are with rare exception highly schematized and limited in presentation. As pointed out in the beginning, they convey considerable information regarding the collecting trunks and the groups of regional lymph glands, but fail, for the most part, we believe, to give any adequate pictures of the finer character and distribution of the plexuses.

Miss Anderson's articles on ovary and tube are complete as regards both text and illustrations. They have the added merit that her morphological findings are correlated with exact data on the reproductive cycle.

Our own incursion into this field is based on an opportunity we have had of injecting the lymphatics of several rhesus monkeys, the reproductive history of two of which was known. Although limited to only a few animals and hence somewhat fragmentary compared to the large series of sow's ovaries and tubes available to Miss Anderson, our injections appear to be sufficiently satisfactory to justify the presentation of our findings in a series of carefully executed drawings. Moreover, no observations exist to our knowledge on the lymphatics of the reproductive tract of subhuman primates, in spite of the fact that they have become in recent years one of the main objects of investigation in the field of reproduction. Finally, upon the basis of quite recent advances in the knowledge of the functional significance of lymphatics and the formation of lymph, as well as from recent investigations of the physiology of reproduction, opportunity is given to present certain ideas, which we considered to be of value, regarding the specific function of the lymphatics supplying the female reproductive tract.

MATERIAL AND METHODS

Our material consisted of the reproductive tracts of four female rhesus monkeys (*Macaca mulatta*). Two of the animals were juveniles which, from their size and the appearance of the ovaries, must have been close to sexual maturity. The previous history of these two was not obtained.

The other two animals were fully mature specimens whose reproductive history was known. For these two macaques we are indebted to Dr. Carl G. Hartman who generously placed them at our disposal. The first (monkey 582) had ovulated regularly and was killed on the twenty-third day of the cycle; its ovaries contained a large corpus luteum. The second (monkey 609) was a few days over 2 months pregnant.

The technique of injecting the lymphatics was as follows. The animals were anaesthetized with ether. The symphysis pubis was split in the midline, the abdomen opened and the reproductive tract entirely exposed. The animal was killed at this point. The vagina was cut open near the ventral midline, from the vulva to the cervix and the edges reflected to expose its interior. Injections were performed by the stab technique by means of a 2 cc. syringe and a 28 gauge needle. The fluid injected was India ink diluted one-half with distilled water. After injection the reproductive tracts were widely excised and placed in 10% formalin. Subsequently parts of the specimens were dissected under a Zeiss binocular dissecting microscope at magnification ranging from four to thirty times. Some of the dissected specimens were then cleared by the Spalteholz method, or sectioned, and stained with haematoxylin and eosin.

Attempts were made to display the lymphatics of the ovaries, uterus, vagina and sexual skin. Not all of the injections were equally successful. Any one familiar with the stab technique for lymphatics will realize that the spread of the injection fluid from the initial bleb into the surrounding lymphatic plexuses is variably successful. In spite of certain failures, our efforts were rewarded by a number of excellent

injections from which the illustrations accompanying this paper were prepared.

By the technique of stab injection one runs a certain risk of injecting veins instead of, or as well as, lymphatics. Consequently the proof that one is dealing with lymphatic channels must be kept in mind. Our criteria for regarding the vessels, which we are presenting, as lymphatics rest on the following considerations: the lymphatics form irregular networks and are consequently much more plexiform than the veins; they also have thinner walls and are more erratic in their course and irregular in contour and diameter than the venous channels. When numerous valves are present, the lymphatics resemble beaded chains. Moreover, the finer lymphatic plexuses often originate from characteristic blind terminal sprouts. Finally, it is usually possible to trace the lymphatics directly into their collecting trunks and thence into the regional lymph glands. All of these differences combine to make differentiation from venous injections possible. In the areas selected for drawings we regard the injected vessels as being exclusively lymphatic, with the exception of the vagina shown in figure 4, in which we are aware from histological study of the sectioned specimen that ink, besides filling lymphatics, was present in slight amount mingled with blood in some of the veins.

Cognizance must also be taken of the possibility of confusing extravasates, which have spread between collagenous bundles in connective tissue or between bundles of muscle fibers, for lymphatics. The stab technique of injecting lymphatics invariably produces an extravasate into the tissue around the site of injection, and a successful preparation depends upon the spread of the injection from this local region into the lymphatics in the fields beyond. An appraisal of whether one is dealing with patterns of extravasation or with true lymphatics depends upon the discrimination of the two conditions by applying the criteria for recognizing lymphatics which were enumerated above. In the present

study only those areas in the specimens were utilized where extravasations were regarded as being absent or at a minimum.

OBSERVATIONS

The results of our study will be presented in reference to a series of drawings and photographs of our injected specimens.

The vagina. Figures 1 to 4 illustrate the lymphatic plexuses of the vagina. Figure 1 shows the entire wall of the vestibule and vagina of a juvenile macaque with the lymphatics injected. The ink gained entry to the lymphatics from two points of injection: first, from the large bleb of ink in the tunica propria of the vestibule (lower right hand corner of figure) and second, from a point of injection near the cervix (upper left of figure). It will be seen how from these sites of injection the ink spreads extensively in the lymphatic plexuses of the mucosa of the vagina and vestibule. The lymphatics of the vestibule are somewhat larger and coarser than the delicate, fine-meshed plexus of the vagina proper; moreover, they lie apparently in a looser type of connective tissue than those in the vaginal mucosa and are consequently more difficult to inject without producing extensive extravasation.

Figures 2 and 3 are drawings at higher magnification of two fields indicated by a rectangle and square in figure 1. It is seen in figure 2 that the lymphatics form a rich, delicate plexus situated in the tunica propria. Numerous short, slightly bulbous terminal lymphatic sprouts appear to connect with the main plexus. In figure 3 the lymphatics of the vaginal part of the cervix uteri are shown. They constitute a delicate plexus similar to the one in the vagina proper. A photograph of a thick histological section of the wall of the vagina is presented in figure 14, showing the character of the injected lymphatics.

Figure 4 illustrates an injection of the lymphatics of the vaginal wall in an adult macaque on the twenty-third day of the menstrual cycle. The field is taken from the posterior vaginal wall where the surface is thrown up into clumps of

conical epithelial papillae. The epithelium clothes groups of connective tissue projections or papillae which extend out from the tunica propria. In the figure the injected lymphatic plexus in these papillae and the tunica propria of the vagina can be seen. The lymphatics constitute a rich network, extending from beneath the surface epithelium, throughout the



Fig. 1 Vagina and vestibule of a juvenile rhesus monkey with the lymphatics filled by injection of India ink. $\times 2\frac{1}{2}$.

tunica propria into the muscular and outer fibrous coat of the vagina where collecting trunks are formed. The lymphatics of the vagina of the sexually mature animal (fig. 4) are evidently much larger, although apparently no more abundant, numerically, than the vaginal lymphatics of the juvenile macaque (figs. 2 and 3).



Fig. 2 Area from the posterior vaginal wall, contained within the rectangle in figure 1, showing the lymphatic plexus of the mucosa in more detail. $\times 37$.

The uterus. The pattern of the injected lymphatics of the uterus of a very nearly mature, juvenile macaque is shown in figure 5. Lymphatics are abundant throughout the uterine wall. The endometrium contains lymph channels which form characteristic arcades occupying the deeper three-quarters of the mucosa. The most superficial layer of mucosa bounding the uterine lumen appears, on the contrary, to be devoid of lymphatics. At the junction of the endometrium and muscularis

(stratum submucosum) there is a dense plexus of lymph vessels.

The myometrium is richly supplied with lymphatics which pass outward constituting an irregular plexus between the interlacing muscle bundles of the thick vascular layer of the muscularis. A photograph of these lymphatics lying between



Fig. 3 The lymphatics of the vaginal portion of the cervix shown in more detail (area of square in fig. 1). $\times 37$.

the interweaving muscle bundles, and accompanying the adventitia of the coiled uterine arteries, is presented in figure 10.

In the place selected for drawing, lymphatics have not been injected in either the outer muscular layer or in the serous membrane. Nevertheless examination of other parts

of the uterus reveals that these layers also contain lymph channels. The outer (supravascular) muscle layer possesses an intrinsic lymphatic network which anastomoses with that of the middle (vascular) muscle layer. At the boundary of

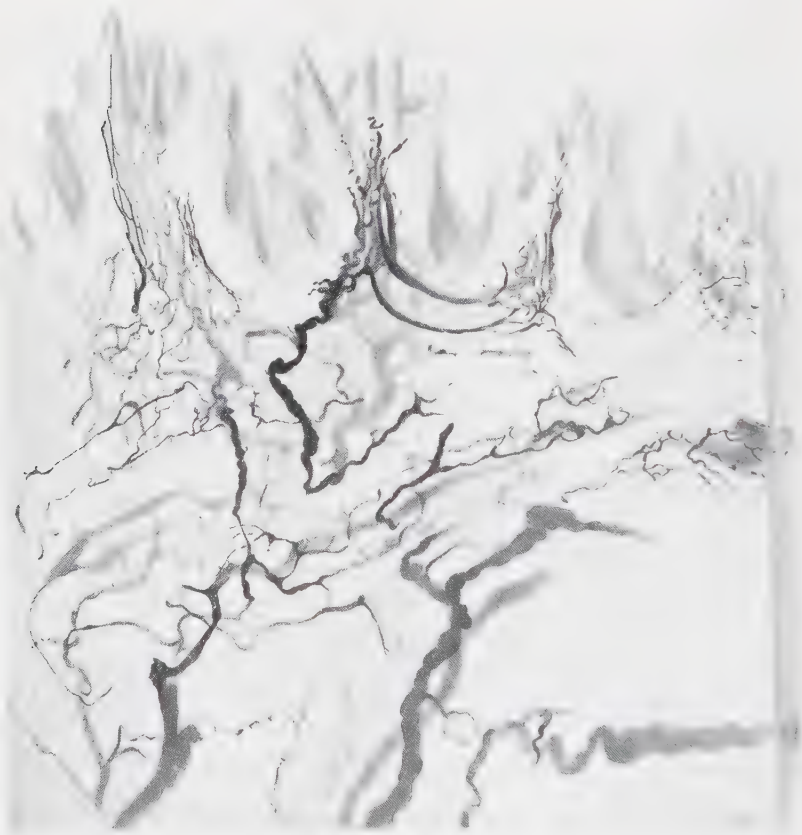


Fig. 4 Injected lymphatics in the vaginal wall of an adult rhesus monkey killed on the twenty-third day of the cycle. $\times 10$.

the middle and outer muscular layers, laterally in the neighborhood of the broad ligaments, these networks unite to form collecting trunks. The beginning of one such collecting vessel is seen on the right in figure 5.

A distinct and separate connective tissue layer of the serosa (subserous layer) is lacking over the entire fundus of the uterus of the macaque, the serosal cells resting essentially, as in man, directly on the outer muscular layer. Consequently in this region there is no special plexus of subserous lymphatics separate from the intrinsic plexus of the musculature. Around the cervix, however, as well as in the parametrium and broad ligaments, a true subserous layer of connective

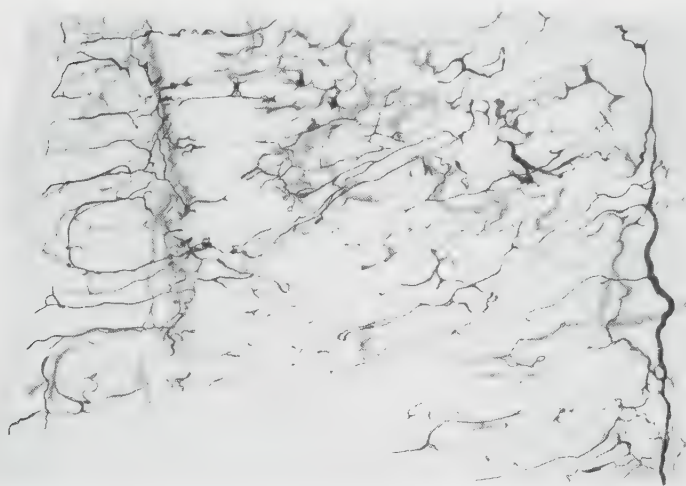


Fig. 5 The lymphatic plexuses in the wall of the uterus of a nearly adult rhesus monkey. The endometrial surface is on the left, the serosal covering on the right of the figure. Notice the rich plexus of lymphatics at the junction of the endometrium and the muscularis. $\times 20$.

tissue is present in which a separate, delicate plexus of lymphatics can be injected.

The gravid uterus. The injection of the lymphatics of a gravid macaque which was a few days over 2 months pregnant turned out very successfully. The results of this injection are shown in figures 6, 7 and 8.

The appearance shown in figure 6 was obtained by dissecting away, over a portion of the body of the uterus, the serosa, besides the most superficial muscle bundles and the accom-

panying lymphatics. The uterus was unopened so that the drawing shows the lymphatics in the middle muscle layer when looked at from the outside under a binocular dissecting microscope. Striking is the wealth and size of the lymphatics. On the right of the figure, near the broad ligament, the lymph channels are seen joining a large collecting trunk, one of several in the neighborhood. These collecting trunks find their way into the broad ligament and parametrium.

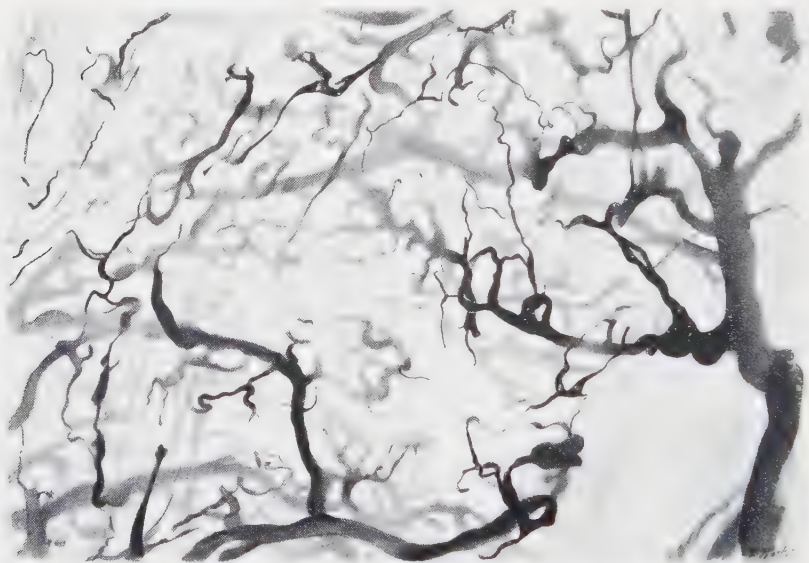


Fig. 6 The lymphatics of the muscularis of the pregnant uterus of a rhesus monkey, seen from the surface, after stripping away the serosa and the outer muscle fibers. On the right a typical collecting lymphatic is shown passing toward the broad ligament. $\times 3\frac{1}{2}$.

After examining the lymphatics of the musculature in the manner described, the uterus was split open, so that the successive layers of the uterine wall and placenta could be observed. Figure 7 represents a cross section through the uterine wall at the site of the placenta. It reveals the lymphatic plexuses in the muscularis and decidua. It is observed that the lymphatics are abundant in the deeper half of the

decidua, the so-called pars spongiosa, whereas the portion of the decidua, designated as the pars compacta, to which the fetal placenta attaches, appears to be devoid of lymphatics. The lymphatics of the spongiosa are very large and numerous and tend to congregate in the loose adventitial sheaths of the coiled endometrial arteries.

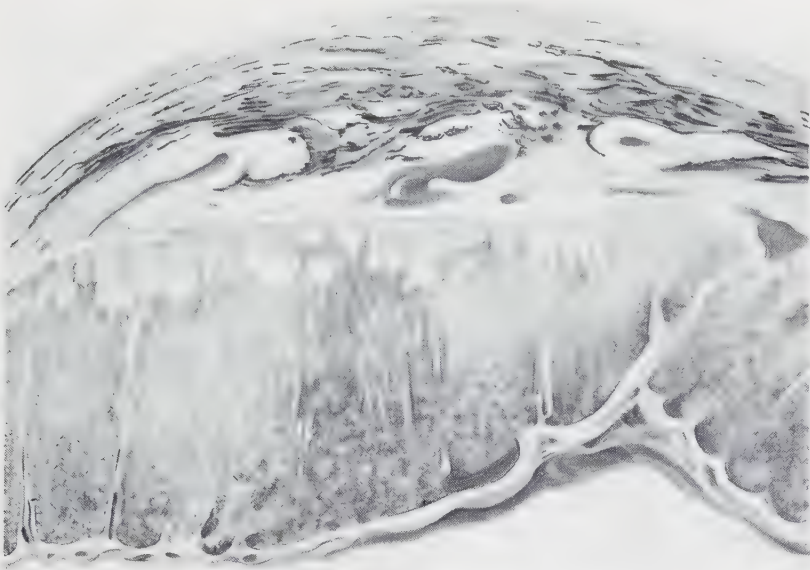


Fig. 7 A free-hand section through the uterine wall and the placental site, showing the plexus of lymphatics in the muscularis and in the spongy layer of the decidua. $\times 6$.

Figure 8 is of a dissection obtained by separating or stripping off the muscularis from the decidua in the region of the placental site. With the muscle removed, one is looking, in the drawing, at the maternal surface of the decidua. In this region of separation of muscularis and decidua one observes a wealth of large lymphatic channels forming a dense, intricate plexus. Lymphatics may be seen in the figure weaving around several thick-walled maternal arteries which supply the placenta.

It will be observed, by comparison of figures 6 and 8 with figure 7, that the lymphatics form flattened bands, very broad and tortuous when seen in surface view, but resembling narrow clefts when viewed in cross section. Figure 11 is a photograph of a histological section through the uterine wall at the placental site. The rich plexus in the basal portion of the decidua, at its junction with the muscularis, is readily

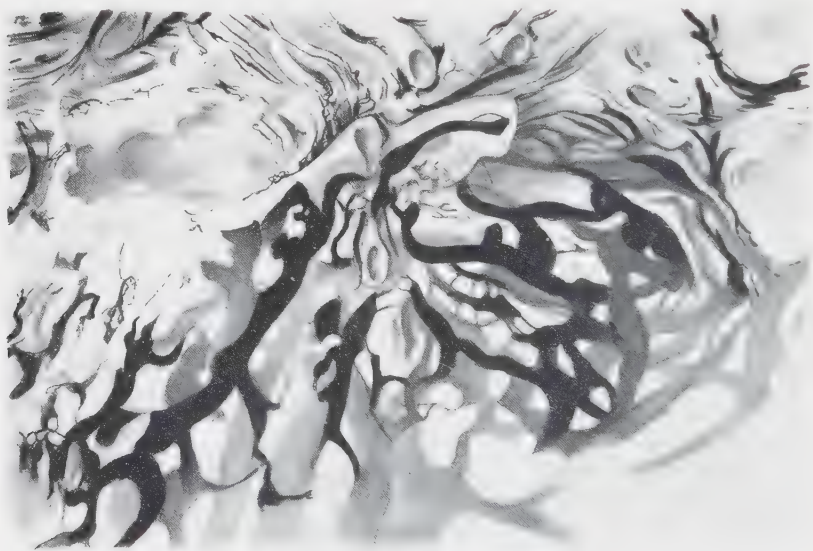


Fig. 8 A view of the rich lymphatic plexus in the decidua, seen after dissecting away the entire muscularis. The lymphatics are abundant in the region of the spiral arteries which penetrate the decidua. Several cut trunks of these arteries are shown in the figure. $\times 5\frac{1}{2}$.

visible. The injected lymphatics are quite sharply demarcated from another set of clefts consisting of the flattened and attenuated uterine glands which are uninjected.

By comparison with the non-gravid uterus, the lymphatic channels of the gestational uterus, although probably not numerically increased, are obviously tremendously enlarged in caliber.

The ovaries and tubes. We did not undertake injections of the fallopian tubes since they are relatively small in the macaque monkey. The lymphatics were injected, however, in two pairs of ovaries. We are not presenting any figures of the ovarian lymphatics, because our limited material does not add essentially to the complete study of Anderson on the sow's ovary. In regard to the ovary of a macaque monkey from the twenty-third day of the cycle containing a large corpus luteum, the following notations were made. The ovary is richly supplied with lymphatics. Delicate lymphatics located in the theca interna surround the small resting, graafian follicles which are present at this stage. In the well-developed corpus luteum, which is perhaps a week to 10 days old, ribbon-like lymphatics are abundantly present in the strands of theca interna which partially subdivide the luteal mass. A plexus of lymphatics is also present in the theca externa which surrounds the corpus luteum. When the intact ovary was examined under the dissecting microscope, a tiny cap of anastomosing lymphatic vessels was seen on the protruding pole of the corpus luteum, whereas the surface of the ovary everywhere else was totally devoid of lymphatics. Examination of the interior of the cut ovary bears out the conclusion that the lymphatics, excepting in the vicinity of the former point of rupture of the follicle, do not reach the surface of the ovary. The lymphatics associated with both corpus luteum and follicles drain away from the surface into the interior of the ovary where they anastomose to form collecting trunks leaving by the hilus.

The sexual skin. The sexual skin was examined with some interest, because the intrinsic lymphatics of this peculiar tissue of certain catarrhine monkeys and apes have never been demonstrated before. It was found that from a bleb of ink introduced into the corium of the sex skin collecting lymphatic trunks could be readily filled. These led invariably, in the subcutaneous tissue of the thigh and groin, to sets of inguinal glands. The intrinsic plexus of the corium of the sex skin proved, on the contrary, difficult to inject. Nevertheless,

repeated trials yielded eventual success. In figure 9 we present a cleared preparation of a piece of the sex skin with the lymphatics injected. This specimen is from our pregnant macaque. The lymphatics constitute a very dense network of relatively

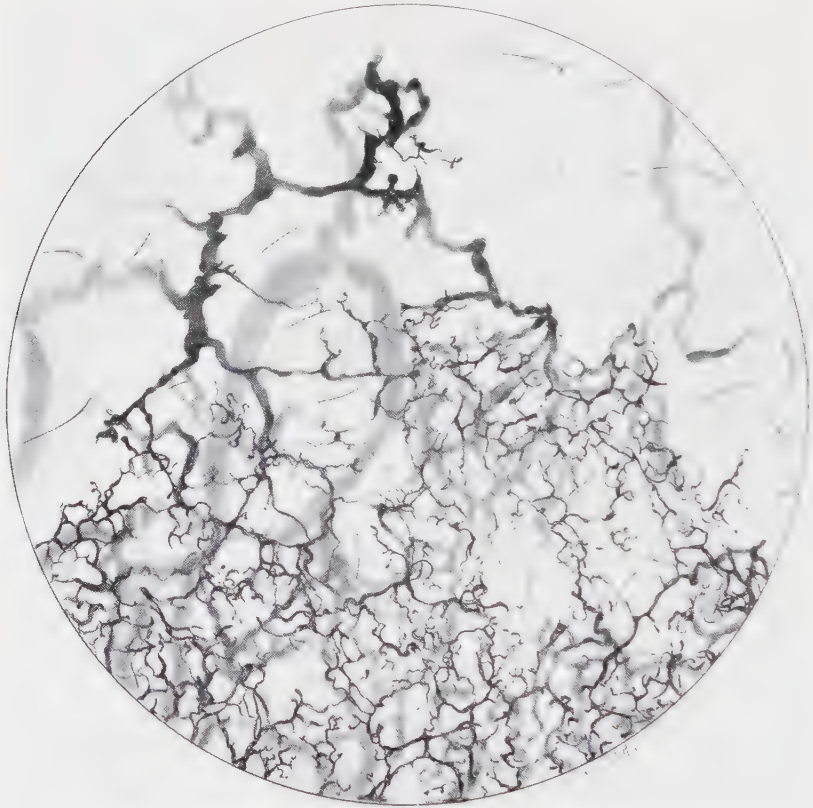


Fig. 9 Injected lymphatic plexus in the corium of the sexual skin of a pregnant rhesus monkey. From the fine-meshed surface network the lymphatics pass into a coarser lymphatic plexus situated beneath in the subcutaneous tissue. The specimen was prepared by Spalteholz's clearing method. $\times 14$.

small calibered lymphatics situated in the corium from which the lymph is drained into larger channels which run in the subjacent subcutaneous tissue. Figure 13 is a photograph of a histological section through the sex skin, showing the injected lymphatic plexus.

DISCUSSION

From the figures it will be observed how extensive the lymphatic plexuses of the female reproductive tract are. Their relative abundance compares favorably, if it does not even exceed, that of other tissues which are known to be relatively rich in lymphatics, for example, parts of the gastrointestinal and respiratory tracts, especially the nasopharynx, the corium of the skin, and the diaphragm.

The question presents itself as to why the female reproductive tract should be so richly supplied with lymphatics. A thought close at hand is that it may be connected in some way with the fact that the reproductive tract undergoes cyclical changes. The studies of Anderson on the sow's ovaries and tubes demonstrate, conclusively, that the lymphatics undergo changes during the cycle. Her observations indicate clearly that the lymphatics of the tube undergo cyclical enlargement in caliber, although probably not in actual number. Injection of the sow's mesosalpinx, ampulla and isthmus was more complete and more readily accomplished during oestrus and the first few days thereafter. The vessels of the ampulla were found, for example, to be twice as large and more readily injectable during oestrus than in the dioestrous interval. Moreover, our observations on the gravid macaque's uterus indicate that during pregnancy the lymphatics of the uterine wall undergo tremendous hypertrophy, a fact also noted by Schick ('06) in the human.

The above remarks taken together with certain other recent observations on the physiology of the female reproductive tract offer, we believe, a clue, hitherto overlooked, to explain the functional significance of the lymphatics of the female reproductive tract. It should be recalled that evidence has accumulated in recent years that around the time of oestrus the ovaries, fallopian tubes, uterus, vaginal wall, and sexual skin undergo, to variable degrees, periodic hypertrophy and swelling. In many parts of the reproductive tract this expresses itself as histologically demonstrable oedema, e.g., the oedema of the decidua at the site of implantation (Wis-

locki and Streeter, '38), the oedematous swelling of the sexual skin in the pig-tailed macaque and baboon (Krohn and Zuckerman, '37; Aykroyd and Zuckerman, '38), as well as the changes in water content of the uterus of the rat, indicated by the study of Astwood ('39). Furthermore, Krohn and Zuckerman ('37), Thorn and Harrop ('37) and others have recently shown that oestrogenic substances have water- and salt-retaining properties. Moreover, it has been demonstrated experimentally that after injection of the sex hormones, certain of the female reproductive tissues become markedly hydrated (Aykroyd and Zuckerman, Astwood).

This circumstance, we believe, explains the results of Anderson on the cyclical changes in the caliber of the lymphatics in the uterine tubes and it accounts for the enormous size of the lymphatics of the pregnant uterus. In tissues which par excellence undergo periodic physiological hydration and exhibit histological swelling and oedema, the lymphatics are presumably of the utmost importance. Were they not abundant and capable of undergoing hypertrophy, the oedema fluid, it might be anticipated, would accumulate and lead to pathological changes. Drinker and his associates ('33, '38) have adduced considerable evidence to show that the composition of lymph is that of dilute blood plasma and that such blood proteins as leave the capillaries to enter the tissue fluid are removed by the lymphatics. Accepting Drinker's concept of the protein content of tissue fluid and lymph, one has to accept, in view of the colloid osmotic pressure of the blood plasma, that the lymphatics provide the principal means for the ultimate escape of the protein-containing interstitial fluid from the tissues. Moreover, Aykroyd and Zuckerman ('38) have reported a high protein content of the interstitial fluid in the active sexual skin of the female rhesus monkey.

In view of these various considerations we advance the interpretation that the abundant lymphatic plexuses of the female reproductive tract and their demonstrated hypertrophy and dilatation subserve the role of reducing the

physiological oedema or hydration to which these tissues are subject, allowing them to return to the resting condition. Other functions which they may exercise are probably quite subsidiary. Miss Anderson, for example, ascribes to the lymphatics of the ovary especially of the corpus luteum, the role of draining the lutein secretory products. Polano ('03) suggests that the ovarian lymphatics act as an elastic cushion, or shock absorber, which prevents injury to the growing graafian follicles, while, just after rupture of a follicle, they prevent the wall from collapsing completely. Polano cites no real evidence to support his concept. Regarding the lymphatics of the tube, Anderson suggests that they may absorb the follicular fluid liberated at ovulation which in the sow may amount to 1.5 cc. Hartman ('32) remarks that the tubal lymphatics are of special interest in connection with the fluid of the bursa and the tubal lumen, since they offer large surfaces for resorption. We regard it as worth mentioning, also, that the uterine lymphatics may participate in the resorption of the fluid which distends the uterine lumen at oestrus in the rat and mouse. Grosser ('19) suggests that the blood vessels are arranged to produce some sort of turgidity of the fallopian tube during ovulation and that the alternate filling and emptying of the associated lymph spaces might act as a sucking mechanism. As Anderson points out, this theory may be discarded when the drainage of the tubal lymphatics is considered. The possible role of the tubal lymphatics in absorbing foreign material from the lumen, mentioned by Anderson, seems to be of doubtful consequence under normal conditions. Similarly the involvement of the lymphatics of the reproductive tract in various inflammatory diseases can be of pathological importance only. However, in tissues which become so readily hydrated, the thought lies close at hand, that in pathological states, with possible occlusion of part of the lymphatics, fibrosis might be an aftermath.

SUMMARY

The lymphatics of the reproductive tract of the female rhesus monkey were injected. Rich intrinsic lymphatic plexuses are demonstrable in the uterus, vagina and sexual skin. In the non-gravid uterus, conspicuous lymphatic networks are visible in both the endometrium and muscularis. In pregnancy these plexuses hypertrophy; the lymphatics of the endometrium, although large and abundant, are limited to the pars spongiosa; the compacta and junctional layers of the decidua, on the contrary, are devoid of lymphatics excepting a few which extend out a short distance in the adventitial sheaths of the arteries entering the compacta. The vagina possesses a very dense net of lymphatics in its tunica propria. Similarly the sexual skin has an extensive network of lymphatics in the corium which connects with a coarser meshed plexus lying in the subcutaneous tissue.

Reasons are advanced for believing that the rich lymphatic supply of the mammalian female reproductive tract is related mainly to the hydration, or physiological oedema, to which these tissues are subjected periodically under the influence of the sex hormones, more especially, estrin. Evidence indicates that the lymphatics are hypertrophied during the active phases of the cycle. The lymphatics serve to dehydrate the tissues by removing the protein-containing tissue fluid and hence promoting return to the resting or dioestrous condition.

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PLATE I

EXPLANATION OF FIGURES

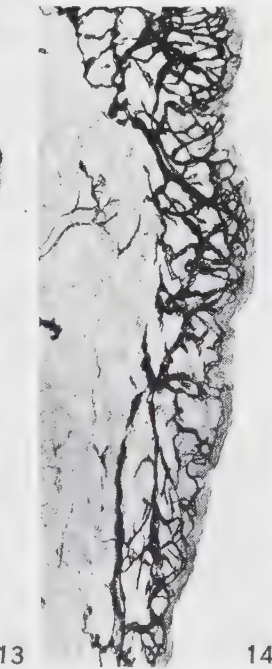
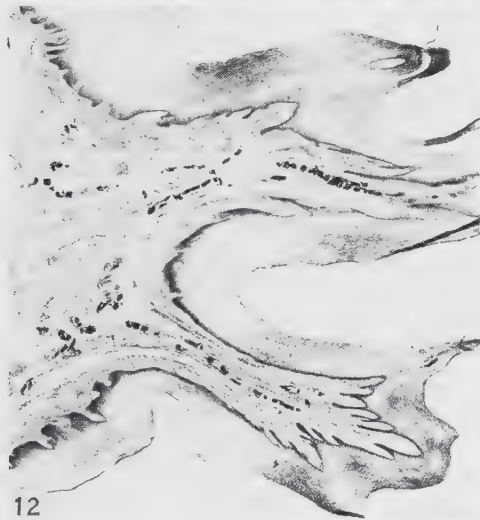
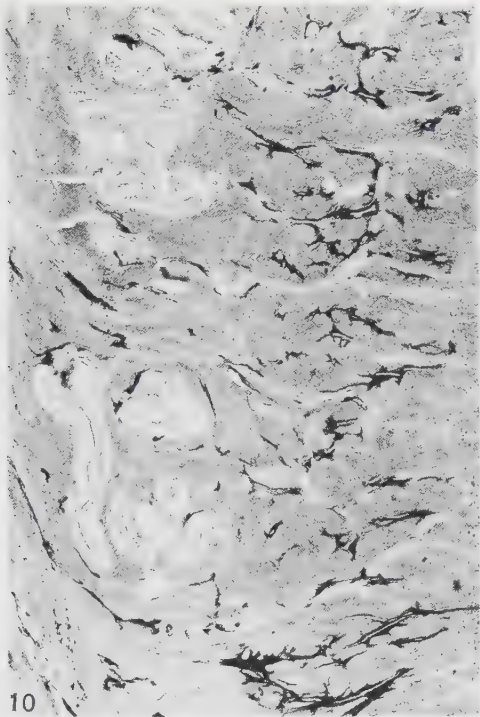
10 Photograph of a histological section of the muscularis of the uterus taken from the specimen from which figure 5 was drawn. The lymphatics injected with ink are seen interweaving with the muscle bundles and blood vessels. $\times 24$.

11 A photograph of the pregnant uterus showing the large lymph channels filled with ink in the deeper part of the decidua. Compare this picture with figure 8. Notice that besides the lymphatics there are other clefts, visible above in the figure, which are uninjected. These are the flattened lumens of uterine glands. $\times 20$.

12 A section through the vaginal wall of a rhesus monkey on the twenty-third day of cycle, showing lymphatics in the tunica propria. Compare with figure 4 which is from the same specimen. $\times 20$.

13 A section through the sexual skin showing the lymphatics in the corium. This and figure 9 are from the same specimen, obtained from a pregnant animal. $\times 24$.

14 Photograph of a stained, thick section of the vagina illustrating the lymphatic plexus in the tunica propria. Same juvenile rhesus monkey as in figures 1, 2 and 3. $\times 24$.



THE PATHWAYS OF ABSORPTION OF SODIUM FERROCYANIDE FROM THE SUBARACHNOID SPACE INTO THE VENOUS SYSTEM

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ONE FIGURE

The absorption of cerebrospinal fluid from the subarachnoid space into the venous system has previously been studied (Weed, '14 b) by replacing the cerebrospinal fluid with a foreign solution. The foreign solution of choice, since the work of Weed ('14 a), has been iron ammonium citrate and a salt of ferrocyanide. This solution on acid fixation yields Prussian blue, and the pathways into the venous system indicated by precipitated Prussian blue have been considered those pathways by which the cerebrospinal fluid escapes into the veins.

This approach to the problem has been criticized by Weed ('35), the originator of the method, and by Flexner ('33) because it does not eliminate postmortem diffusion and consequently it may give a false picture of the avenues of escape of cerebrospinal fluid from the subarachnoid space. In view of this criticism, a method utilizing a modification of the Altmann-Gersh ('32) technique has been devised to eliminate postmortem diffusion. This procedure and the observations which it has yielded are presented below.

MATERIALS AND METHODS

The animals used in these experiments were young adult cats and dogs. They were anesthetized with ether given by

¹ Henry Strong Denison Scholars for 1938-1939.

tracheal cannula. This anesthesia had been found to give constant pressure levels of the cerebrospinal fluid (Weed and McKibben, '19; Weed and Hughson, '21).

An isotonic (2%, 0.24 normal) solution of sodium ferrocyanide containing 0.1% calcium chloride was used for irrigation of the subarachnoid space; this solution was found by trial to be non-toxic, the criteria being that the animal exhibited no abnormal neurological signs while under the ether anesthesia or upon recovery from the anesthesia. Upon the addition of ferric chloride, the ferrocyanide was precipitated readily as Prussian blue, giving clearly defined crystals for histological study. Since isotonic sodium ferrocyanide was non-toxic and readily precipitated, it was the solution of choice in all the experiments.

Injections were made into the cisterna magna through a glass manometer, by means of which the cerebrospinal fluid pressure was measured. Injection fluids were introduced at a pressure (5 to 10 mm. of saline) in excess of the normal pressure for the particular animal under observation. Pressure was kept at a constant level by adding fluid to the manometer to compensate for that absorbed into the subarachnoid space. In order to insure a sufficient quantity of precipitated Prussian blue in the midsagittal sinus for study, it was necessary to inject at least 5 cc. over a $3\frac{1}{2}$ hour period. Following such an injection, samples of the urine were taken and tested for Prussian blue. If the urine failed to give a positive test, the injection was continued until a positive reaction was obtained; or if the test remained negative, the animal was discarded.

Two series of experiments were performed, one of which was designated acute, the other chronic. In the acute experiments the animals were anesthetized and irrigated without any previous preparation. In the chronic experiments a sterile operation was performed on the animals 3 months previous to the injection. This operative procedure consisted of removing the calvarium, then covering the exposed dura mater

by suturing the muscles, fascia and scalp in the order named, after which the animal was allowed to recover completely. Since the dorsal plates of the skull had previously been removed, it was necessary at the time of injection only to incise the scalp in order to expose the superior sagittal sinus and surrounding tissue. By this method no handling of the brain was necessary; thus any possible pressure changes and injury to the leptomeninges which might arise from opening the cranial cavity were eliminated.

Upon exposing the brain, a small, metal, rectangular form was placed lightly over the superior sagittal sinus. This form was then filled with liquid air, which immediately froze the sagittal sinus region in situ. After the tissue was thoroughly frozen, a block $1 \times 1 \times 1.5$ cm. was removed with a sharp wood chisel. The block of frozen tissue was then transferred to a vacuum tube maintained at about -20°C . (Gersh, '32). The tissue was left in this tube until it was thoroughly dried. The block was then infiltrated in 52°C . paraffin in a vacuum tube for 20 minutes, removed, and placed in a paraffin oven for 16 hours.

The block was embedded and sectioned in the usual manner. Sections 10, 15, and $20\ \mu$ thick were mounted dry on the slide and passed rapidly through a xylene solution of anhydrous ferric chloride into a saturated solution of hydrated ferric chloride in absolute alcohol. The slides were then transferred directly to a saturated aqueous solution of ferric chloride for about 15 seconds. This step was essential for complete and well-defined precipitation. From this aqueous solution the slides were washed in absolute alcohol, xylene, and then mounted in balsam (Gersh and Stieglitz, '34). The ferric chloride reacted with the sodium ferrocyanide to precipitate Prussian blue. The slides were immediately studied microscopically in order to determine the position of the precipitated Prussian blue crystals. This precaution was necessary as the crystals tended to fade after standing. As a check in determining the histological structure, alternate serial sections were stained in haematoxylin and eosin.

OBSERVATIONS

In both the acute and the chronic experiments no untoward symptoms were observed if the pressure of the irrigating solution was only 5 to 10 mm. of saline greater than normal cerebrospinal fluid pressure.

Gross inspection of the tissue after paraffin infiltration showed no distortion of structure. The dura mater remained in position, and there was no evidence of separation. The chronic experiments differed from the acute only in that there was a layer of fibrous tissue about 2 mm. thick over the top of the dura; this was attached to the dura alone and had not caused any adhesions between the meninges and the brain itself. The midsagittal sinus was in all instances completely filled with blood.

Microscopical examinations of the sections with a low-power objective (fig. 1) showed no displacement of structure. The meninges had their normal relations, and the trabeculae in the subarachnoid space were intact. In the chronic experiment the connective tissue which had grown over the dura was well delimited to that position, and in no place was the relationship between the meninges disturbed.

The precipitated Prussian blue was confined to the subarachnoid space. The arachnoid villi, so well described by Weed ('14 b), were numerous throughout the midsagittal sinus (fig. 1) and the precipitated crystals were observed in great numbers in these outpouchings of the arachnoid membrane. There were also slight aggregations of Prussian blue around the majority of the veins lying in the limited portion of the subarachnoid space studied here, which was within a few millimeters of the midsagittal sinus (fig. 1), and along the falx cerebri. A comparison of the amount of Prussian blue in the arachnoid villi and in the arachnoid veins showed a far heavier precipitation per unit volume in the villi than in the veins. In no place was the blue observed to have diffusely stained the leptomeninges or the walls of any of the arteries. No crystals were observed in the perivascular spaces. This is in accord with the studies of Weed ('14 b) in which he

demonstrated that, providing a normal pressure relationship was maintained between the arachnoid and the venous system, there was no evidence of the passage of a foreign solution from the subarachnoid space along the vascular channels to the cerebral capillaries.

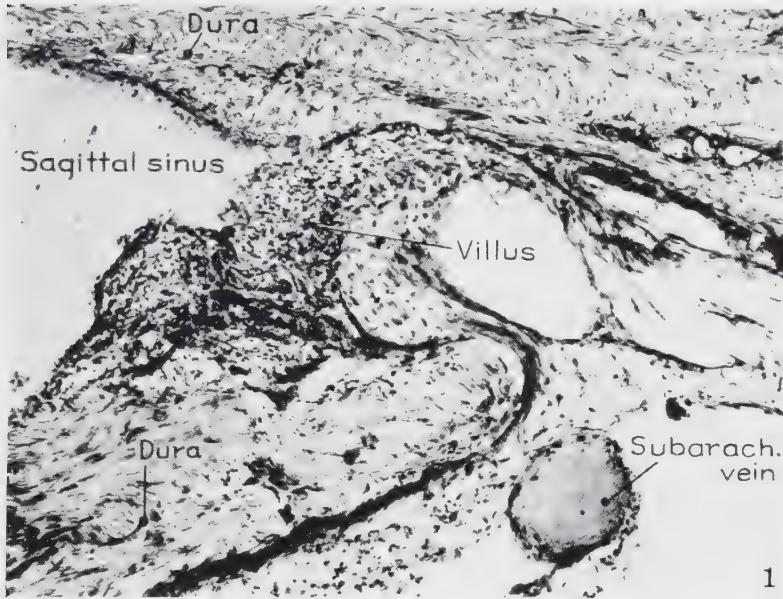


Fig.1 Photomicrograph ($\times 97$) of a section of the superior sagittal sinus, stained with haematoxylin and eosin. On the right the dura has been invaded by a large cellular villus which is protruding into the sagittal sinus. This villus is seen as an outgrowth of the loose cellular tissue of the arachnoid membrane. Non-stained sections which were passed through ferric chloride solutions showed large crystalline accumulations of Prussian blue in this villus. These crystals extended all the way from the subarachnoid space through the villus up to the blood in the sagittal sinus. Also shown is one of the subarachnoid veins which showed precipitation of Prussian blue in its wall.

The above observations were confirmed with a high-power objective. In addition, it was found that the accumulations near and in the arachnoid villi could be traced through the endothelial cells lining the venous sinuses. The accumulations around the veins of the subarachnoid spaces were enmeshed

between the mesothelial cells, which surrounded these vessels, and could be traced up to, and in the endothelial cells lining their walls. These veins were extremely thin-walled, and studies made with connective-tissue tissue stains did not reveal any muscle in their walls.

DISCUSSION

This study of the pathway of absorption of the cerebrospinal fluid was made in an attempt to eliminate the criticism of postmortem diffusion. Since this process must take place by means of water, it would seem that the procedure of freezing the tissue *in situ* and dehydrating at -20°C . would greatly diminish, if not eliminate the possibility of postmortem diffusion. The only contact of the tissue with water was an immersion of the sections in a saturated aqueous solution of ferric chloride for 15 seconds to complete the precipitation of Prussian blue. During such a short period it was felt that no appreciable amount of diffusion would occur, and if there were diffusion, it would be much less than that produced by methods in which the tissues were fixed for hours in an aqueous medium.

Although the method was not designed to be quantitative, the observations showed that there were far greater quantities of the precipitated crystals in the arachnoid villi than in the subarachnoid veins. These observations in no way contradict the conclusions of previous investigations (Weed, '14 b) that the primary pathway of absorption is by way of the arachnoid villi. In addition, however, they bring evidence that a secondary avenue of escape is through the arachnoid veins.

Previous histological studies (Benninghoff, '30) on man have shown that there are no muscle fibers in the veins of the subarachnoid space. This observation was also made in our study on cats. Since these veins are anatomically different from the veins in most of the other parts of the body, a possible explanation may be found for the passage of the sodium ferrocyanide through these vessels.

SUMMARY AND CONCLUSIONS

The pathways of absorption of cerebrospinal fluid into the venous system have been studied by means of a method designed to eliminate postmortem diffusion. The observations lead to the following conclusions:

1. An isotonic solution of sodium ferrocyanide introduced into the cerebrospinal fluid passes from the subarachnoid space into the venous blood channels by way of the arachnoid villi and the subarachnoid veins. An individual villus shows a far greater concentration of Prussian blue than an individual vein.

2. Normally no crystals were observed in the perivascular spaces or in the walls of the arteries of the subarachnoid space.

3. The walls of the veins passing through the subarachnoid space in cats and dogs are without muscle.

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ANTERIOR PITUITARY CHANGES FOLLOWING ADRENALECTOMY IN THE RAT

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THREE PLATES (NINETEEN FIGURES)

Numerous publications deal with the changes in the anterior pituitary after gonadectomy and after thyroidectomy. Much less, however, is known regarding the response of this gland when the adrenals have been removed.

The previous reports pertaining to this problem fall into two general categories. One series of reports deals with the pituitary in Addison's disease, while another treats the condition of the pituitary in experimental adrenal ablation.

It has been noted by Shumacker and Firor ('34) that the results of pituitary studies in Addison's disease are more clear and consistent than have been the reports upon experimental animals. Kraus ('23, '26, '27) described in his cases of Addison's disease a most consistent diminution in the number and size of the acidophil cells of the anterior lobe, and also found distinct alterations in the basophil picture. There was a marked scarcity of well-granulated basophils. We quote from Kraus' report ('26) his observations on the basophil group:

“ . . . Die meisten basophilen Zellen zeigten Kernpyknose, überreichliche Körnerelimination, Umwandlung in eine wabige, fast zusammenfließende Masse, Kleinheit und unregelmässige Form des Zelleibes, mangelhafte Körnelung und

¹ Aided by a grant from the Board of Research of the University of California. Assistance was rendered by the Works Progress Administration, Project No. 10482-A5.

undeutliche Zellgrenzen." Further, ". . . In einem Fall von Morbus Addisoni sah E. J. KRAUS im Vorderlappen ein Bild, das einem zirrhotischen Prozess mit Regeneration des Parenchyms und zwar der basophilen Zellen glich."

The chief cells were increased at the expense of other cell forms and these also sometimes showed nuclear pyknosis.

Berblinger ('32) also observed a diminution in normal basophils in Addison's disease. Crooke and Russell ('35) noted in the pituitaries of patients with Addison's disease a decrease in the number of acidophils. They state, however, that the reduction in acidophils is "very variable," and is "far less than the reduction of basophil cells." These workers noted an interesting series of changes in the basophil group and state that "constant features are the extreme reduction of the basophil cells and the presence of a series of abnormal basophil transitional cells." They described vacuolation of the cytoplasm, pyknosis of the nuclei and 'granular agglutination' in these basophil transitional cells. Crooke and Russell also noted considerable numbers of large chromophobes in the glands of their series.

In describing a case of Addison's disease, Severinghaus ('36) observed:

"There were many granulating and degranulating cells, especially among the basophils, and the cupping of cells, the Collin phenomenon, could be seen in almost any cell cord. Where cells are increasing and decreasing in size with great frequency, the increased cupping of other cells would be expected. Addison's disease in general seems to be associated with a depletion of basophils from the hypophysis, but certain glands have appeared to show an attempt to attain normalcy, a process which may be of such short duration as to be generally missed. Basophilic activity seems indicated."

This report is especially important in the light of the present study.

In rats suffering from adrenal ablation, Igura (quoted by Berblinger) and Lehman ('29) have noted a diminution in the acidophils and a corresponding increase in the number of chromophobes. Oka ('37 a, b), also working on the rat, noted

a numerical decrease in acidophils as well as a diminution in their size. Oka noted that the basophil picture was altered and that these cells showed evidence of loss of granulation.

Grollman and Firor ('35) in their studies on chronic adrenal insufficiency reported that basophils were diminished in the pituitary of the dog, but that in the rat the diminution of these cells was not so marked as in the dog. "However, the staining of these basophilic cells was very abnormal."

MATERIAL

We have chosen the male rat as the form upon which to execute this study. The ready availability of this animal, the ease of bilateral operation and the fact that pituitary cytology in the rat is exceptionally well known, all combine to make it the animal of choice. We have employed the male because we felt that the cyclic phenomena observed in the various stages of the oestrous cycle in the female might cause some confusion.

It is well known that older animals seem to succumb less readily to adrenal extirpation than do immature rats. For this reason we have employed animals of such an age group that although sexual maturity had been attained we felt that there would still be a high degree of sensitivity to deprivation of the adrenal cortex. Our series of animals consists of male rats between the ages of 65 to 90 days. In the majority of cases, a litter-mate control was provided for each experimental animal and was sacrificed at the same time.

We proposed to deal only with the doubly adrenalectomized animal, since we felt that any observations upon the partially operated rat might be inconclusive if not actually confusing. Since we were interested in the pituitary in acute adrenal insufficiency, we have constantly attempted to sacrifice the animal shortly prior to the presumptive hour of its demise.

It was difficult to estimate the exact hour at which any given animal in adrenal insufficiency might be expected to die. It was necessary to acquire considerable acquaintance with the signs of adrenal insufficiency in the experimental animal, and experience in the interpretation of their severity. Care-

ful weight and temperature data were kept as were observations on other criteria.

Since the general symptoms of adrenal insufficiency have been adequately described for a number of forms, we do not feel that their enumeration need be repeated here (compare for the dog, Banting and Gairns, '26; rat, Pencharz, Olmsted and Giragossintz, '31).

We have always attempted to estimate the degree of severity of symptoms shown by our animals at the time of autopsy. We have chosen to indicate an animal showing a minor degree of insufficiency as one having a 'one plus' reaction. If the animal's symptoms were moderately severe but had not reduced it to a terminal state, the condition of the animal was designated as 'two plus.' If the animal showed such symptoms that it appeared to be approaching a terminal state, the reaction was indicated as 'three plus.' In no case was the pituitary studied if the animal had died of adrenal insufficiency. Post mortem changes were thus ruled out.

The present observations are based upon the effects of total adrenal ablation on forty-eight experimental animals. In some of them, death would unquestionably have come in a period of minutes or at the most an hour or two. Other animals would doubtless have survived for a much longer period. No animal was included which failed to show such definite evidence of adrenal insufficiency that eventual death was inevitable (i.e., no animals in the series reported here were sacrificed while showing what was judged to be a 'one plus' reaction).

METHODS

Our routine method of treatment has been Zenker-formol fixation followed by the Mallory-azan staining method as adapted to the pituitary of the rat by Koneff ('38). The details of this method have been thoroughly stated in the original description and in a subsequent paper (Koneff, '39).

With this, as with other methods, we have always attempted to maintain a minimum time between the death of the animal

and fixation of its pituitary, and to observe adequate precautions in handling.

The application of more than one technique, however, in such a study as this, was considered highly desirable. It is true that as a general stain, the Mallory-azan method as modified by Koneff gives an adequately clear differentiation of tinctorial types in the anterior lobe; it even gives us some conception of the distribution of mitochondria in the basophils, and one can estimate to a degree the activity of the Golgi apparatus by the appearance of its negative image.

As has been previously noted by one of us, Severinghaus has emphasized the fact that confusion of acidophilic granules and mitochondria may lead to serious error. Although what are unquestionably mitochondria are apparent in the basophils in the Mallory-azan preparations, due probably to Zenker-formol fixation, these bodies are not as distinctly seen nor their form as well preserved as when Champy fixation, post chromation, and a specific mitochondrial stain are employed.

For the purpose of demonstrating mitochondria in the various cell types, we have employed the Altmann-Masson technique essentially as it has been described in the various publications of Severinghaus. The use of this method enables us to distinguish mitochondria from acidophilic granules very adequately in most instances. Differentiation of the mitochondria in the other cell types is a relatively simple matter.

It is necessary, furthermore, if we are to obtain a sound estimate of the functional state of the various cell types, that studies of the Golgi apparatus be made. In this laboratory, we have used various methods in the past for the demonstration of this organelle in the anterior pituitary, and have discarded all but the eminently satisfactory Kolatchev method as modified by Nassonov.

Sections for studies on mitochondria and on the Golgi apparatus have been made in paraffin at 3 to 4 μ respectively, while for the Mallory-azan preparations the celloidin technique was used, sections being cut at 4 μ .

We found that counterstaining some of the sections showing the Golgi apparatus with the Mallory-azan stain, as used in the guinea pig by Dr. Hadley Kirkman (personal communication) was a very useful method.

RESULTS

Acidophils

When individual cell types were considered, the most striking alteration was in the acidophil picture. This is partially due to the fact that the acidophils are normally the most conspicuous cell type in the anterior lobe and any significant change in them will be immediately noted.

The characteristic alterations in the histological and cytological picture of the acidophils can be enumerated as follows:

1. There is an obvious decrease in the number of acidophils.
2. The size of the acidophils is markedly diminished.
3. Accompanying the decrease in cell size, there is observed a gradual decrease in granular material. The number of cells showing diminished granulation is increased as compared to the normal gland. Many of the acidophils appear as small cells with just a rim of granules surrounding the nucleus.
4. No evidence of restoration of granular material in the acidophils is detectable.

The following table is a summary of a statistical analysis of the alterations in the acidophils of the experimental animals in comparison with the findings in the normal controls. The normal control animals constitute one group while the experimental animals are subdivided into groups on the basis of their symptoms at the time of autopsy. The figures represent averages of the acidophils in each group of animals.

*Acidophilic cells of the anterior pituitary*¹

	SEVERITY OF SYMPTOMS	SIZE	A	B
Normal control group		152.6	64.6	35.4
Experimental group	++	115.3	51.0	49.0
Experimental group	+++	107.3	48.6	51.4

¹ Explanation of table.

Size = area occupied by the camera lucida tracings of 100 cells measured with planimeter and expressed in units (one unit being equal to 10 sq.mm.).

A = acidophils tightly packed with granular material.

B = acidophils showing various degrees of degranulation.

In no single animal of the normal group was the size of the acidophils so small as those in the individual experimental animals (152 units as compared with 128 units). In no instance was the percentage of these acidophils, showing plentiful tightly packed granules, as great in the experimental animals as in the controls (62% as compared with 53%).

5. Special methods reveal the following changes in the cell organelles of the acidophils in the experimental group. The Golgi apparatus is decreased in size and becomes a small compact structure applied to the nucleus or closely adjacent to it (figs. 7, 8, 9, 10). The mitochondria are diminished in number and size and are far less prominent than in the acidophils of the normal animal.

The smaller size of the cells and the diminution in granulation would lead one to suspect diminished function. This impression is confirmed by recourse to preparations designed to show mitochondria and the Golgi apparatus in the various cell types.

Severinghaus ('33) has characterized the acidophil type of Golgi apparatus in the rat as "a basket-like network closely applied to one side of the nucleus." The association of the Golgi apparatus of this cell group (acidophil and acidophilic chromophobe) with the nucleus is certainly a characteristic phenomenon in the rat hypophysis. Severinghaus is undoubtedly correct in referring to it as being "typically restricted as a cap to one side of the nucleus." It is noteworthy, however, that in the normal gland the interanastomosing strands of this cap often surround the nucleus for a considerable distance and that the apparatus may occupy no inconsiderable portion of the cytoplasm on one side of the nucleus (figs. 5 and 6). The changes in the Golgi apparatus in the acidophils of the adrenalectomized animal are regressive. The apparatus shows marked diminution in size, contraction, and finally becomes a small compact osmiophilic spherule in close approximation to the nucleus. In some instances, the Golgi apparatus may retain its normal elongated shape, but the size is much reduced and it appears as a black line next to the nucleus (fig. 8).

When one studies material designed to show differentiation of mitochondria and acidophilic granules, one gains the impression that the mitochondria in the acidophils of the adrenalectomized animal are certainly less prominent than in the normal control. The mitochondria of the normal acidophil are ordinarily spherical, sharply outlined, are often of considerable size, although they vary; rod forms are scarce. In contrast, the acidophils of the experimental animal show mitochondria as granules of lesser size and number and with an indistinctness best described perhaps as a general loss of prominence. Those acidophils which retain an approximately normal appearance show mitochondria whose form, size and distribution are within the limits of normal variability.

On the basis of this data, one seems justified in assuming that the acidophil undergoes regressive changes after adrenalectomy and that the functions of this cell type are diminished.

It is obvious that a significant proportion of the acidophils gradually lose their granular material as the severity of symptoms progresses. Very many of them ultimately become exceedingly small cells with a limited amount of cytoplasm containing only a rim of granules surrounding the nucleus. This loss of granulation and diminution in cell size progress simultaneously.

There is furthermore no evidence of new granule formation in the cells thus affected; and the regressive state of the Golgi apparatus and the mitochondria, in preparations designed to show them optimally, indicate a marked degree of inactivity on the part of the acidophils.

As one examines a typical Nassonov-Kolatchev preparation of the anterior pituitary of the experimental group, the marked preponderance of small agranular cells with an acidophilic type of Golgi apparatus, as compared with the normal gland, clearly indicates that many of the acidophils have reverted to a permanent chromophobic state. Although these regressive changes are pronounced, we observed no evidence of actual degeneration in this cell group.

Basophils

Although, as already noted, the changes described in the acidophils are particularly conspicuous, further examination reveals that there are also changes in the basophils and that these changes are profound. These alterations should be prefaced by a brief review of the characteristics of the basophils of the normal male rat.

A description of the basophils when the Mallory-azan technique is used, after Zenker-formol fixation, has been given by Koneff ('39). We quote from this description:

"Two groups of basophils were distinguished, light and dark. The latter have a considerable amount of granular or flocculent basophilic material which makes the whole cell appear dark blue in color, while the light basophils consist of cells containing a considerably smaller amount of fine granular material suspended in a light blue basophilic cytoplasm. These light blue cells are usually rounder in shape, larger and apparently in different stages of degranulation or dissipation of granular material."

Further, "Severinghaus ('33) has already mentioned as a normal variant of the basophils, a cell with 'vacuolation' of the cytoplasm and has warned the reader not to confuse this vacuolation with that found in the formation of castration cells. This type was occasionally observed in our sections of normal glands." Moreover "occasionally even the typical signet ring (Schleidt) basophils characteristic of old castrates, are present in normal animals though the percentage is low (1.5%)."

We feel that in the light of the present study it is necessary to describe another basophil variety, also rarely seen in the normal gland (not exceeding 3% of all basophils). The characteristic of this basophil is that the cytoplasm appears in clumps and is often distributed about the periphery of the cell.

It should be clearly understood that we imply no actual classifications of the basophil group such that any of the cell varieties mentioned fall in a distinctly separate category from any other. Intermediate stages and gradations between these varieties can be seen.

When a specific mitochondrial method is used, the radiation of the mitochondria from the region of the Golgi apparatus and their increase in size as we progress peripherally from that region can be clearly noted. These points have been discussed in the literature and in this work have been abundantly confirmed. It is a difficult task to assign to a particular variety of basophil characteristic mitochondria, since granules, short rods and filaments may be seen in basophils of any category. We have the impression, however, that the lighter type of basophil ordinarily will show granules of somewhat smaller size than those seen in the dark polyhedral variety, and that their dispersal is more general. Finer filaments and rods seem more frequently found in this cell than in the dark type. In the darker basophils we have the impression that the mitochondria are usually prominent granules or rods. Any hard and fast rule, however, cannot be made.

The numerous reports upon the basophilic type of Golgi apparatus which have appeared in recent years make any protracted discussion of this organelle in the normal gland unnecessary. We agree with the conception that it is an invaginated, double-walled sphere. In our observations upon the Golgi apparatus in both the glands of normal and adrenalectomized animals, we have made parallel studies on the positive picture as revealed by osmium impregnation and on the negative image of the Golgi apparatus (macula) as shown by our general histological method.

Even a preliminary examination of the basophils in this series of adrenalectomized animals revealed that, as has been noted by Grollman and Firor ('35) and Oka ('37), there was considerable variation from the normal picture. Although we accumulated no elaborate statistical data it was obvious that the total number of true basophils in most of the experimental animals was reduced. The first impression gained was that there had been a reduction in the size of most of the basophils, both the nucleus and cytoplasm being involved. Recourse to measurement of camera lucida outlines with the planimeter

showed that there was a definite and constant reduction in the size of both the nucleus and cytoplasm. Such an investigation has revealed that the decrease in general cell size is constant. Decrease in cytoplasmic area seems definitely correlated with the estimated degree of severity of the animals' clinical symptoms. The following figures represent averages of the basophils in each group of animals indicated.

Basophilic cells of the anterior pituitary

	SEVERITY OF SYMPTOMS	CELL SIZE	SIZE OF NUCLEUS
Normal control group		296	70.7
Experimental group	++	247 }	60.2
Experimental group	+++	232 }	

This table also shows that the size of the nucleus is reduced in the experimental animals. It is noteworthy that there was no overlapping in figures. In no case was the average cell size or nuclear size so great in the experimental animals as in the normal controls.

No less important than these gross changes in the basophil picture are the alterations in the internal organization of these cells. In the glands of experimental animals after both types of fixation and after application of appropriate staining methods, it was obvious that in many of the basophils the usually fairly uniform distribution of the granular material had been profoundly disturbed.

In material stained by the Mallory-azan method there were formed in the cytoplasm of some of the basophils, clumps or agglomerates presumably derived from the granular content, which had previously been uniformly dispersed. These clumps manifested a tendency to become concentrated at the periphery of the cell. Some times this clumping formed aggregates of relatively great size and as a result of the peripheral concentration of such material, we found about the nucleus a lighter zone in which some basophilic content was distributed in a reticular fashion, or such material might be completely absent about the nucleus (fig. 14). This resulted in either a

very light or absolutely colorless area in the perinuclear region. The reader will note that we have described similar cells as rare components (not more than 3%) of the basophils of the normal gland. In glands of experimental animals such cells are definitely increased in number and may make up as many as 10% to 22% of the total number of basophils.

This clumping and peripheral distribution of the cytoplasmic content of the basophils does not always present the exaggerated picture which we have just described. In many cells the agglomerates of basophilic material are less coarse and show less tendency toward peripheral concentration. These cells, nevertheless, do not present a normal cytoplasmic pattern. The cytoplasm is shredded or the basophilic material is massed together in small clumps. These shreds or clumps, not so strongly basophilic as in those cells where the clumping is very marked, are separated by clear spaces giving the cytoplasm a spongy appearance. Such cells are especially well demonstrated after Champy fixation. Shredding of the cytoplasm and the assumption of a spongy appearance may progress to such a degree in certain cells that the degenerative character of the alteration is unquestionable. Such cells, although often small, may sometimes attain a very great size (fig. 17). We feel that the cytoplasmic changes described are expressions of regressive changes. One has the feeling that where the clumps of basophilic material are large and deeply stained, such a transition has occurred in the dark type of basophil; whereas in those cells in which basophilic material is lightly stained, less abundant, and more evenly dispersed, the cell affected is probably the basophil of the light variety.

In certain basophils the regressive changes are primarily expressed in the character of the cytoplasm (clumping of basophilic material and shredding of the cytoplasm). In other basophils, however, one may find severe disturbances in the nucleus without accompanying alterations in the cytoplasmic pattern even though the volume of the cytoplasm be greatly reduced. Such nuclear changes may present all degrees of the disintegrative process.

Further evidence of nuclear alteration, not of such an obvious pathological character as that mentioned above, is present in the basophil group.

In the normal gland the shape of the basophilic nucleus is usually round or oval, while in the glands of adrenalectomized animals there has been noted an increased tendency toward elongation. Camera lucida tracings demonstrated that the elongate type of nucleus in basophils in the adrenalectomized animals might reach a figure as high as 23% of the nuclei of all such cells, as compared with 13% in the normal controls. These figures are based upon group averages.

It was also found that approximately 10% of the nuclei in the basophils of the experimental animals showed folding of the nuclear membrane—a phenomenon rarely observed in the normal gland.

In those basophil cells in the experimental material which showed frank evidence of degenerative change, there were found deviations from the normal appearance and distribution of the mitochondria. These changes may be characterized as follows: in contrast to a copious distribution of these bodies, which under normal circumstances quite uniformly radiate from the region of the Golgi apparatus, we see in certain degenerating cells in the experimental material what we feel is definite evidence of restriction of these bodies to the Golgi area. It appears as if the normal process of dispersion had been inhibited. In basophils of this kind, mitochondria are almost always granular. Other cells of this variety show reduction in number of mitochondria, and in cells that appear otherwise badly damaged, grouping and, finally, clumping of mitochondria into amorphous masses is sometimes seen.

As will be described in the following paragraphs, a large proportion of the basophils appear to be in a hyperactive state, especially if the Golgi apparatus be taken as a criterion. In these cases it is our opinion that the mitochondria are as prominent as in the basophils of the normal gland or even more prominent. It is our impression that the filaments and

prominent rod-like mitochondria usually described in normal active cells were often found in the basophils of the adrenalectomized animal which showed other evidence of hyperactivity.

Such findings could be generally confirmed in preparations stained by the Mallory-azan method. Although this method is not especially designed to show mitochondria and though there is, of course, no differentiation between mitochondria and acidophilic granules, there can be no question that in the basophils the mitochondria often stain fairly adequately with azo-carmin in the Mallory-azan preparation after Zenker-formol fixation. This fact was utilized in correlation of the cytological findings obtained in the two staining methods described.

In our observations on the Golgi apparatus, we have used two methods of study. With our general histological method (Mallory-azan) it has been our practice to study and ascertain the size of the negative image of the Golgi apparatus in relation to the nucleus of the same cell. In this way, even with the Mallory-azan stain, we have been able to form an impression regarding increased or decreased cellular activity as based upon the size of the Golgi apparatus.

To obtain an idea of the degree of reticulation and changes in form and size, we have always used, parallel with such studies positive impregnations of this organelle. We felt that such studies could leave little doubt as to whether there were changes in the experimental material when the Golgi apparatus was used as a criterion.

After a preliminary study we found it convenient to divide our experimental series into two groups: namely, one representing the glands of animals which had survived for a relatively short period and in which the symptoms were usually of moderate severity only, and another group in which the animals had survived for a longer period and in which symptoms were much more severe at the time of autopsy.

Even a preliminary examination of the Golgi apparatus in the basophils of the first group mentioned above showed very

definitely that this organelle was either very markedly larger than one finds in the average of basophils in the normal gland, or very considerably smaller.

When the Golgi apparatus was of the larger type it was often very large, highly reticular, and formed often a fine meshwork throughout the greater part of the cytoplasm (compare fig. 11 with figs. 12 and 15). The typical oval or spherical shape was lost and ramifications pushed their way into the cytoplasm to an amazing degree. Such a Golgi apparatus would of course have an indistinct outline if its negative image only were considered. In animals whose symptoms had reached only a moderate degree of severity this hyperactive Golgi apparatus was present in approximately 30% of all basophils.

We noted in the Mallory-azan preparations, and especially in cases where symptoms were of moderate severity or short duration, numerous cells which had a basophilic type of macula, often of a kind indicating stimulation. These cells often had a large light vesicular nucleus and an irregular shape. They tended to occupy the available space between surrounding cells and often seemed partially to engulf them. Such cells might be classified on the basis of their tinctorial reaction as chromophobes. However, they are certainly of basophilic origin. All this is confirmed by our findings when a Nassonov-Kolatchev preparation is counterstained with the Mallory-azan method. This is a cell type in which the very elaborate Golgi network previously described is often observed. We consider that these cells show marked evidence of stimulation and that they are basophils in the last stages of granular depletion. We do not imply that these cells are the only members of the basophil group showing evidence of stimulation as revealed by the Golgi apparatus, for we have observed hypertrophy of this organelle in other varieties of basophils.

In contrast to these cells showing a stimulated Golgi apparatus, the apparatus in many of the remaining basophils was revealed as a small, compact body without any ramifications (fig. 13).

If it is considered that the size of the nucleus in the basophils of the experimental animals was somewhat reduced, it can be easily understood that the Golgi apparatus being much smaller than the nucleus, was remarkably smaller than the average size of that apparatus in the basophils of the normal gland. The degree of contraction of the Golgi apparatus in some cells progressed to such an extent that this structure appeared as a minute, densely impregnated body.

Interestingly enough, the Golgi apparatus was visible even in cells which had undergone a considerable degree of degeneration.

Moreover, this very small Golgi apparatus was usually associated with other criteria, indicating a lessened degree of cellular activity. It was common in cells showing nuclei whose shape was irregular and elongate, whose cytoplasm showed diminution of granular material or the signs of cytoplasmic disintegration as described in the preceding paragraphs (figs. 17, 18, 19), and the aberrations in the mitochondrial picture previously noted.

In animals where symptoms were of greater severity and where the post-operative course was longer, the basophils showing this small inactive Golgi apparatus were increased at the expense of those showing evidence of stimulation. The basophils showing a very elaborate network were obviously decreased in number.

The cells in which the Golgi apparatus was increased in size far beyond ordinary limits were found to show all degrees of granulation and depths of staining. One had the impression that these basophils were very active from a secretory point of view.

In the other group of basophils, with the small Golgi apparatus, reduced prominence of mitochondria and cytoplasmic disorganization, one had the impression that secretory activity was considerably decreased, if not entirely absent.

This latter opinion would seem to be confirmed by the fact that some of these regressive cells seem to revert to the true chromophobic state while others undergo degeneration in both

cytoplasm and nucleus and become minute elements, the recovery of which seems highly improbable. It is worthy of note that even though these cells are on the borderline of disappearance from the histological picture, nevertheless the mitochondria and Golgi apparatus, though regressive and aberrant, can often be discerned. It is apparent that very many basophils do not lapse into a state from which there is an easy route of return to functional activity. It seems, also, illogical to suppose that they may become the usual basophilic chromophobe (fig. 16).

Chromophobes

As pointed out in the discussion of the acidophil changes, the most striking alteration in the chromophobes is the increase in the acidophilic type. The evidence for this point rests upon the study of preparations designed to show the Golgi apparatus. In the classification of anterior lobe cells other criteria than those usually considered, namely, granular content and the tinctorial reaction, should be taken into account.

We should not overlook the fact that certain of the basophils in our material reach a degree of granular depletion in which they may have no discernible content, and on the basis of tinctorial reaction with the Mallory-azan technique, might be included in the chromophobe group.

As has been previously pointed out, cytological investigation reveals the fact that these cells can be looked upon as basophils in the last stage of granular depletion.

DISCUSSION

An obvious question is whether the changes in the anterior pituitary cells described here are the result of a specific interrelationship between the adrenal cortex and anterior hypophysis, or whether they are indirect results, i.e., come from alterations occurring in some other gland of internal secretion or in other physiologic mechanisms.

The decreased size of both the chromophil cell types would suggest perhaps that altered fluid balance operating in such a way as to reduce the total tissue fluids might be responsible for some of these changes.²

As a matter of fact there is no way of ruling this factor out of consideration.

We have very definite reasons, however, for believing that the entire picture cannot be explained on this basis. These reasons may be enumerated as follows:

1. All cells are not affected in the same degree and in many of them the evidence of volumetric decrease is slight or absent.

2. A cell or cells showing profound regressive changes are found in juxtaposition to those whose appearance is normal or which show evidence of great stimulation.

3. The histological and cytological evidence is such that simple reduction in size cannot explain all the phenomena we have observed in these cells. This evidence points clearly in the opposite direction, namely, that the diminution in granular content and regressive changes in the cell organelles are correlated with this decrease in cell size.

When we consider the evidence for retarded function in the acidophils we cannot but reflect upon another glandular deficiency in which a similar picture has been described, namely, ablation of the thyroid.

It is now a well-established fact that, in animals deprived of the thyroid, active acidophils tend to disappear from the anterior pituitary. Severinghaus, Smelser and Clark ('34) reported that the loss of acidophilia on thyroidectomy in the rat was "due to degranulation of the acidophils and to the small size of many of the cells." They also stated that "certain acidophils are greatly enlarged but show a vacuolation which closely approximates the degenerating vacuolation of

²Grollman and Firor ('35) reported a dilatation of the capillaries in the pituitary of dogs in adrenal insufficiency and state that the same phenomenon was observed in rats, although in the latter this finding was less striking. We have noted congestion and capillary dilatation in our experimental material (compare figs. 1 and 2).

the acidophiles described by Trautmann in the thyroidectomized goat."

Although we have not observed enlargement and vacuolation of the acidophils in the adrenalectomized animal, we felt that the regressive picture of the acidophils might be correlated with changes in the thyroid.

Examinations of the thyroids of these animals and comparison with littermate controls showed uniformly some evidence of underactivity in the thyroid in experimental animals. In general, the thyroids of the experimental group showed a lower epithelium, evidence of increased colloid storage and less prominence in the clear vacuoles seen in the follicular colloid, than was observed in the normal controls.

Grollman and Firor ('35) reported "The adrenal cortical hormone is without the least effect on the growth of rats stunted by chronic adrenal insufficiency . . .," while "administration of the growth hormone of the anterior pituitary is accompanied by growth which approximates or exceeds that observed in normal animals." They concluded that "the cessation of growth in animals maintained for long periods on chronic adrenal insufficiency is not due to lack of the adrenal secretion, but is due to pituitary insufficiency induced secondarily by the adrenal insufficiency."

Our findings as regards the profound changes in the acidophils in acute adrenal insufficiency would seem to explain the diminished output of growth hormone from the pituitary.

The evidence for production of the growth hormone by acidophils is well known and from the changes in the acidophils in the adrenalectomized animal we are willing to advance the postulate that growth hormone in such glands would be proved to be significantly reduced were they to be analyzed by the implant method.

We have stated that even in the absence of differential cell counts it is obvious that the total number of basophils in the experimental animals was reduced.

A clue as to what has become of them lies in the fact that regressive and in many instances definitely disintegrative

changes in a significant proportion of the remaining basophils is seen. Reduction in cell size, the nuclear changes already described, disorganization of the normal cytoplasmic pattern, an extremely small Golgi apparatus in many instances, reduction in the prominence of mitochondria and in some cases clumping of these bodies into amorphous masses all indicate functional regression or even cellular destruction. We believe that our findings may be correlated with the reports of Kraus, Berblinger and others who have reported changes in the basophils in cases of Addison's disease.

It was a somewhat surprising fact that of the basophils remaining in the gland of the adrenalectomized animal, a very large proportion showed some evidence of stimulation. Especially was this manifested in the Golgi apparatus which was sometimes very elaborate and showed a marked degree of arborization; this usually correlated with normal or increased cytoplasmic volume, normal mitochondria and a healthy appearance of the nucleus.

Perhaps this state of affairs might be best expressed by saying that in this group of chromophils the various grades of cellular activity seen in the normal gland are lacking and the basophils are either decidedly inactive from a secretory point of view, or they show evidence of increased activity. Cells representing a mean between these two extremes are less noticeable than in the normal gland.

In interpretation of this fact we may say that we are unconvinced that there is adequate evidence, on cytological grounds at least, for subdividing the basophils into groups responsible for the elaboration of this or that hormone.

It seems to us a more reasonable interpretation that the signs of increased activity seen in so many of the basophils in the adrenalectomized rat are compensatory in nature and that these cells are simply making a last attempt to supply a deficiency due to the failure of their companion cells to function adequately. A similar opinion has been advanced by Severinghaus regarding the significance of increased basophilic activity in his case of Addison's disease.

Additional evidence to support this point of view lies in the fact that the hyperactive basophils are notably reduced in animals with very severe symptoms. This would indicate, we believe, that there is an increasing tendency toward failure of this compensatory mechanism.

It is interesting, furthermore, that in our material we have found large hyperactive cells of the basophilic group which with the Mallory-azan method might be considered chromophobes. One might presume that these are the counterpart of the large chromophobes described by Crooke and Russell ('35) in Addison's disease.

The nature of the changes in the basophils would lead us to suspect some very definite changes in the hormonal content in the gland. Shumacker and Firor ('34) reported "The hypophyses of adrenalectomized rats show a decrease in gonad-stimulating power." Such data correlates very nicely with our findings that the changes in the basophils indicate progressive decrease in function. Such a speculation is based upon the widely accepted evidence that the basophil is responsible for the elaboration of at least a significant part of the gonadotropic complex.

Whether or not the other hormones, thyrotropic, lactogenic and adrenocorticotropic, are produced by acidophils or basophils, the concentration and elaboration of these hormones in the glands of adrenalectomized animals would doubtless be diminished, since both chromophil types show evidence of diminished function and damage (even though some basophils are hyperactive).

SUMMARY

I. The histological and cytological changes seen in the anterior pituitary in male rats showing acute symptoms after double adrenal ablation are:

A. Diminution in number and size of acidophils. This decrease in number and size is correlated with progressive loss of granular material, although no evidence of actual degeneration is seen in this cell group.

B. Regressive changes in the Golgi apparatus and in the mitochondria clearly indicate diminished activity in this group of cells.

C. Obvious diminution in the number of basophils is observed, together with diminished size of both the cytoplasm and the nucleus.

D. Cytological changes in the basophil group are correlated with the degree of severity of symptoms and with the length of the post-operative period.

These changes in the cytology of the basophil fall into two categories: The majority of the basophils reveal a small inactive type of Golgi apparatus, indistinct or aberrant mitochondria which accompany degenerative changes in the nucleus and cytoplasm. Many of the remaining show an enlarged Golgi apparatus and prominent mitochondria and a healthy nucleus. Especially is this true where the symptoms of the animal have been of a milder degree of severity or the post-operative period relatively short.

E. Chromophobes in the adrenalectomized animals are definitely increased. As far as can be judged without precise statistical methods, this is primarily due to the reversion of acidophils to the chromophobe state.

II. Although the gland shows degeneration in many basophils, the presence of a significant number of hyperactive basophils suggests that the latter represent an attempt to compensate for the degenerative changes seen in the other basophils.

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PLATE 1

EXPLANATION OF FIGURES

Mallory-azan stain. Photographs were taken with 8 mm. objective; magnification 380 (reduced one-fourth).

1 Field selected from anterior pituitary of a normal male rat. Note general size and distribution of acidophils. Note the normal vascularity.

2 Field selected from anterior pituitary of an adrenalectomized male rat. Note changes in size and granulation of the acidophils. Note also the increased number of chromophobes and increased vascularity.

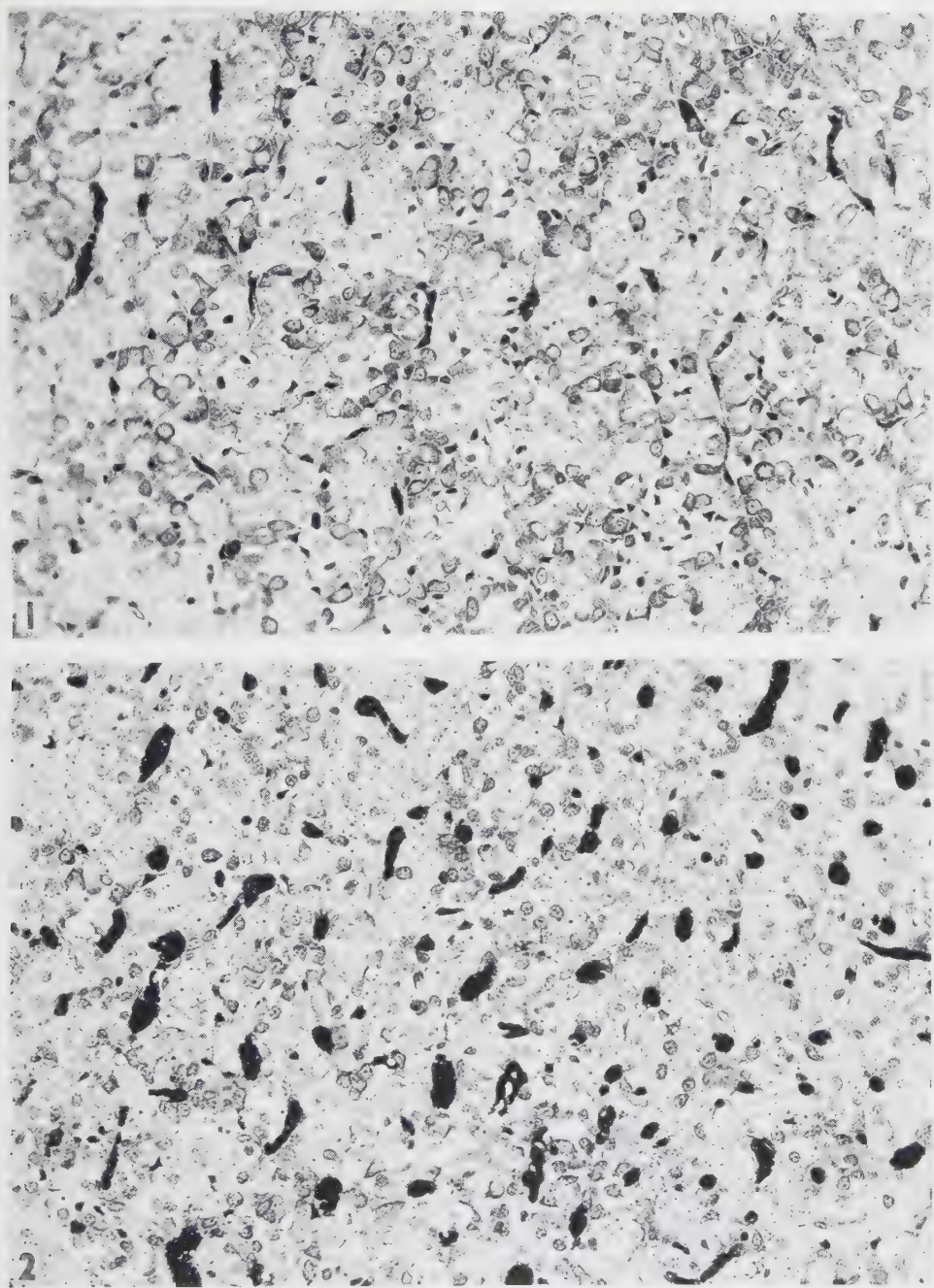


PLATE 2

EXPLANATION OF FIGURES

Nassonov-Kolatchev preparations. Photographs were taken with 5 mm. oil immersion objective; magnification 750 (reduced one-fourth).

3 Selected field from anterior pituitary of normal male rat 70 days of age. Note number of acidophils as well as their size. Note the size of the Golgi apparatus in both acidophils and basophils.

4 Selected field from anterior pituitary of male rat autopsied 9 days after adrenalectomy. Animal was 70 days old at time of autopsy. Symptoms were of moderate severity. Note decrease in well-granulated acidophils and reduction in the size of these cells. Note decrease in size of acidophil Golgi apparatus. Note the greatly enlarged Golgi apparatus in certain of the basophils.

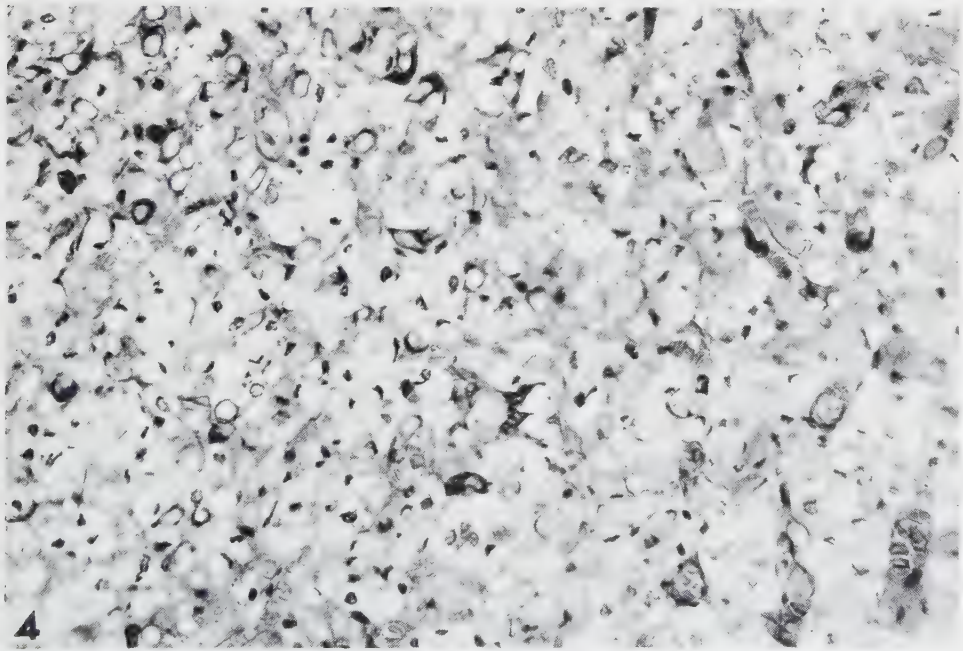
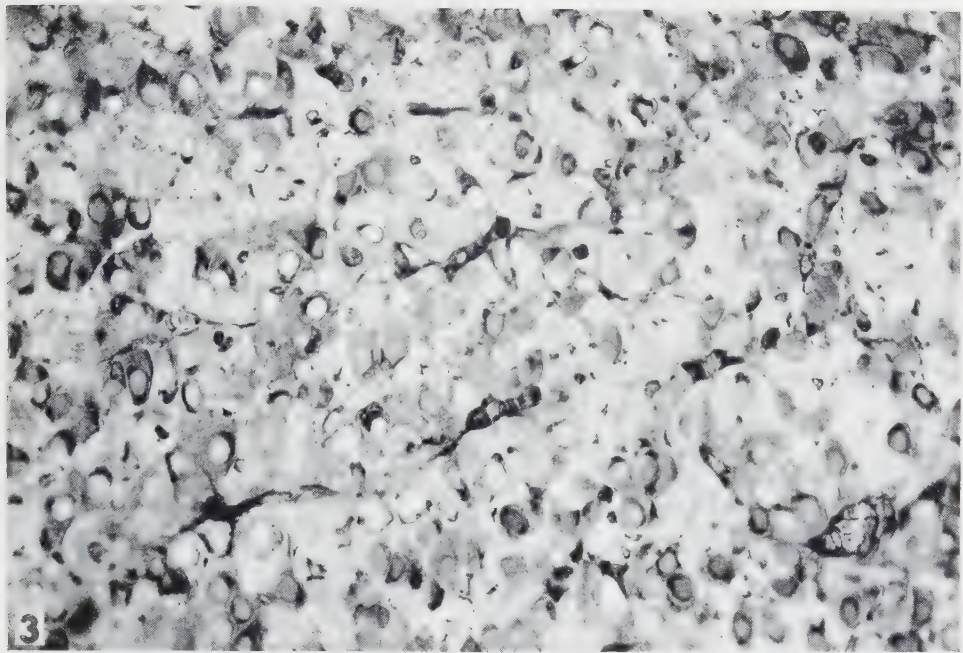


PLATE 3

EXPLANATION OF FIGURES

All photographs were taken with a 2 mm. oil immersion objective. Magnification $\times 1750$ (reduced one-fourth).

5-13 Nassonov-Kolatchev preparations.

14-19 Mallory-azan stain.

5-6 Acidophils from pituitary of normal rats. Note the cell size, size and shape of the Golgi apparatus and the relation of this apparatus to the nucleus.

7-8-9-10 Acidophils from pituitaries of adrenalectomized rats. Note the decrease in cell size and changes in size and shape of the Golgi apparatus.

11 Basophil from pituitary of normal rat. Note general shape and size of Golgi apparatus and its relation to the nucleus.

12-13 Basophils showing the change in size and shape of the Golgi apparatus seen in certain of these cells in the adrenalectomized animal.

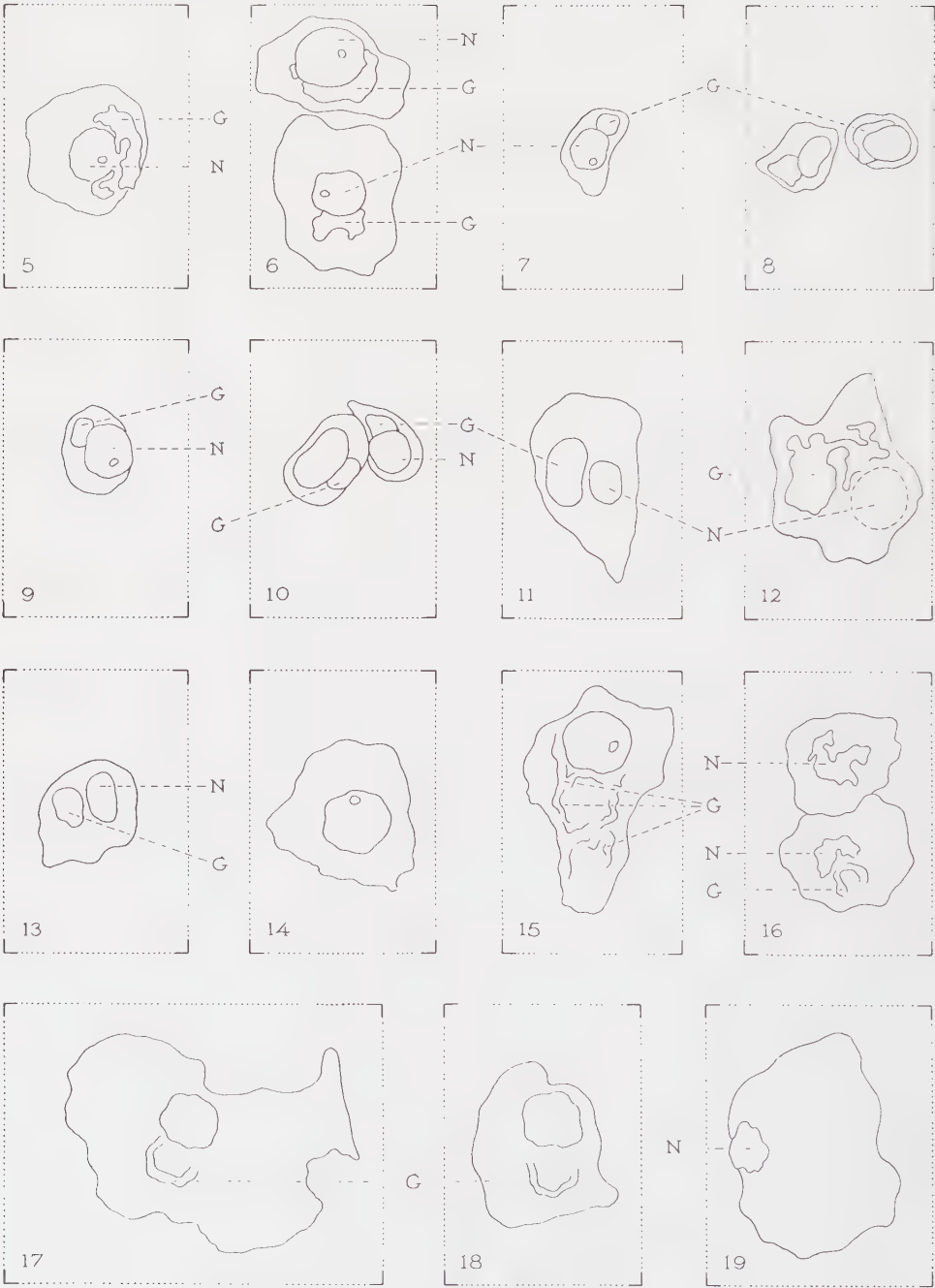
14 Basophil showing clumping and peripheral concentration of the basophilic material.

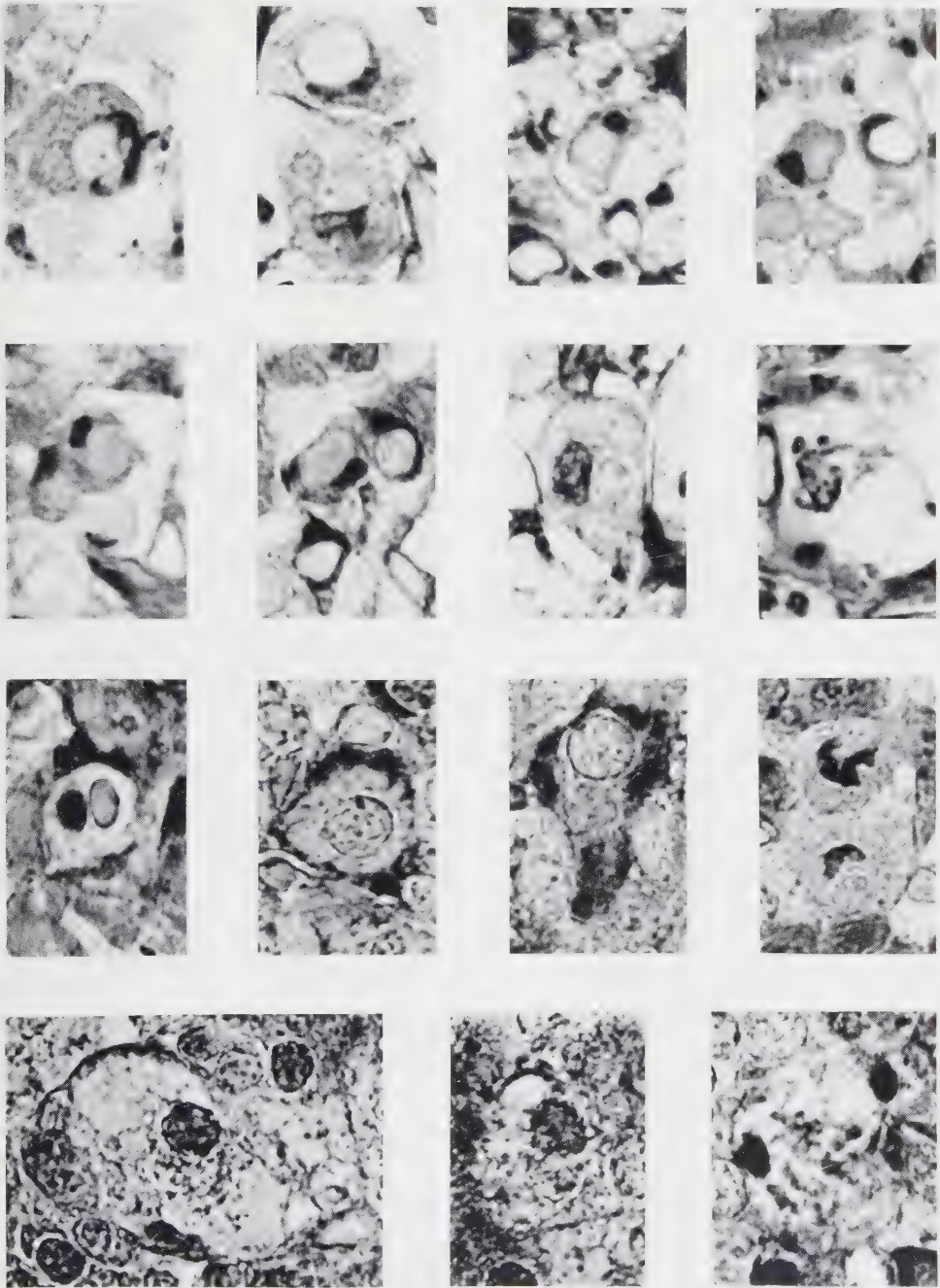
15 Basophil showing enlarged negative image of the Golgi apparatus.

16 Basophils showing degenerative changes in the nucleus and aberrant negative image of the Golgi apparatus.

17-19 Basophils showing degenerative changes in the cytoplasm and progressive degeneration of the nucleus. Note that in figure 17 the degenerative changes are accompanied by a significant increase in cell size.

PITUITARY AFTER ADRENALECTOMY
 J. D. REESE, A. A. KONEFF AND M. B. AKIMOTO





COLLATERAL CIRCULATION FOLLOWING AN OBSTRUCTION OF THE ABDOMINAL AORTA

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ONE FIGURE

The case described in this paper exhibits a pattern of collateral circulation following obstruction of the human abdominal aorta the counterpart of which has not been found in a search of the literature. Similar arrangements of the minor collateral channels have been observed by many workers, but none of them has emphasized the mesenteric circuit which is of major importance in the specimen in question.

Obstructions of the aorta occur most commonly in the region of the ductus arteriosus and the descending arch, and are usually of developmental origin. They may occur also as the result of pathologic processes, or be instituted by operative intervention. Few of the reported cases have any direct bearing upon the present one except to indicate its relative rarity. Enlargement of the mesenterics has been reported in human cases by Deroin (1870) and by Vaughan ('22); also in experimental animals by Pirogoff (1838) and by Sčelkunow ('29). Most of these authors and also others¹ who have investigated the problem ascribe the chief role to other vessels, particularly to the internal mammary-epigastric and the intercosto-lumbar anastomoses. Obviously an important factor in governing the selection of vessels for collateral channels has

¹No attempt will be made to review the literature on the subject. The reader is referred especially to the papers by Tillaux et Riche ('01), Hesse ('21), Abbott ('28), and Sčelkunow ('29), all of which give extensive bibliographies.

to do with the location and extent of the obstruction to the aorta. Only in one of the above-mentioned cases can these conditions be accurately compared with those in the present case. That instance is described by Sčelkunow ('29). The aorta of a dog was ligated between the origins of the renal arteries, and at autopsy a most striking enlargement of the colic anastomosis was found (loc. cit., S. 560, Abb. 10).

The case of obstruction of the aorta described here was that of a male negro, age 32, who died following the removal of a toxic goitre. There was nothing in his history which suggested any vascular disturbances. During a routine dissection, the cadaver was found to have an obstructive lesion of the aorta just distal to the orifices of the renal arteries (fig. 1). From this level to a point 2 cm. proximal to the origin of the inferior mesenteric artery (IM) the vessel wall was irregular, nodular and for the most part stony-hard. The obstruction proved to be due to a calcification which seemed to have developed in aneurysmal dilatations of the aorta. Two of these dilatations were in the anterior wall of the aorta just distal to the renal vessels. A third and larger one was present in the posterior wall slightly below the first two. The point of complete obstruction to the aortic lumen lay between the first two and the third, just at the level of the second pair of lumbar arteries. Each dilatation contained a large organized blood clot and its walls were almost completely calcified. In the regions immediately above and below the dilated areas there were considerable deposits of calcium in the aortic wall, but elsewhere the vessel and its branches appeared to be completely devoid of any changes. Upon gross inspection there was no indication as to the etiology of the lesion.

The most striking change in the vascular arrangement was the pronounced enlargement of the colic anastomosis. The superior mesenteric artery (SM) and its middle colic branch (MC') were almost as large as the aorta, and joined directly with the equally enlarged left colic branch (LC) of the inferior mesenteric artery (IM). Other channels of collateral circulation were the somewhat enlarged and tortuous internal

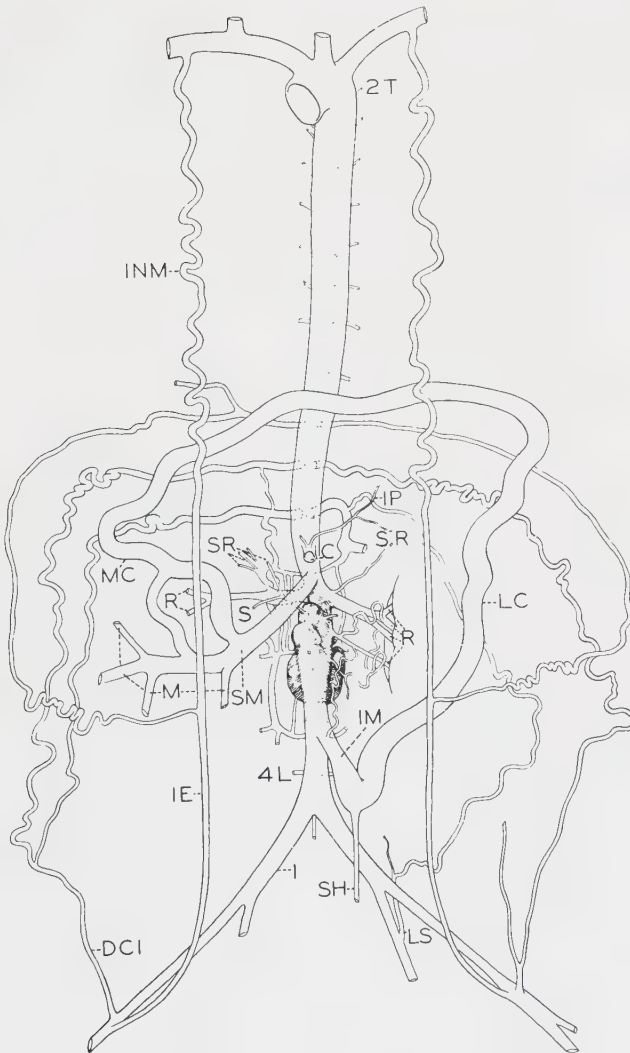


Fig. 1 Arterial anastomoses in a case of obstructed abdominal aorta. Shaded area, calcified aneurisms. Abbreviations: C, coeliac; DCI, deep circumflex iliac; I, iliac; I.E., inferior epigastric; IM, inferior mesenteric; INM, internal mammary; IP, inferior phrenic; LC, left colic; LS, lateral sacral; 4L, fourth lumbar; M, mesenteric; MC, middle colic; R, renal; S, spermatic; SH, superior hemorrhoidal; SM, superior mesenteric; SR, suprarenal; 2T, second thoracic.

mammaries (INM) which joined the inferior epigastric arteries (IE) on either side. The last three pairs of intercostal arteries were enlarged and tortuous and made numerous connections with other vessels. These and other connections shown in the figure were undoubtedly important, but certainly were subordinate to the anastomosis between the two mesenterics.

Situated within the area of calcification, but proximal to the point of closure of the aortic lumen, were the orifices of the spermatic vessels (S), of the second pair of lumbar, and of two small accessory renals on the left side.

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REVIEW

THE EARLY LOOPING OF THE ALIMENTARY CANAL IN THE MAMMALIAN AND HUMAN FOETUS AND THE MECHANISMS ASSUMED TO BE ACTIVE IN THIS PROCESS

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ONE FIGURE

In a work on the formation of the umbilical cord and the umbilical region of the anterior abdominal wall Wyburn ('39) also touched upon the interesting problems of the origin and reduction of the physiological umbilical hernia. His account, however, may be supplemented with the views of these important embryodynamical processes to which I have come after extensive comparative embryological studies of man and other mammals (Enbom, '37, 1 and 2; '38, 2). I shall therefore briefly outline in English the most important results of my earlier works as well as of a recent work on the development of the shape and position of the alimentary canal in mammals (Enbom, '39). It may be premised that among other innovations for these investigations a new plastic method of reconstruction has been elaborated, in which use is made of plastiline plates instead of wax plates (Enbom, '37, 1; '38, 1).

The part played by the vitelline artery, a. omphalomesenterica, in the retraction of physiological umbilical hernia in the opossum, *Didelphys virginiana*, was dealt with in my earlier works ('37, 1 and 2). Respecting the mechanism behind the early reduction of the umbilical hernia in this primitive mammal it was there shown that the omphalomesenteric artery is greatly shortened in connection with the replacement of the umbilical loops. Thus it was established that the stretch of the artery in question from the aorta to the point at which it leaves the mesentery at the flexure of the primary umbilical loop (x in fig. 1) is contracted to only one-half its former length in this its mesenteric portion; that is, the last-mentioned point (x) is drawn

dorsally to a corresponding extent, and the most ventral section of the umbilical loop comes to lie within the aperture through which the hernia protruded, in spite of the fact that the dorso-ventral diameter of the abdominal cavity has not become larger (compare fig. 1 a and b). At this stage—in about 1-cm.-long embryos—the intestinal system is still in a simple phase of development; the small intestine is folded together in a characteristic manner in two groups of loops that pass arcuately around the vitelline artery. In consequence of the shortening of the vessel trunk contained in the mesentery these intestinal loops are tightly compressed, displaced in a dorsal direction and drawn into the abdominal cavity, but otherwise there is no change

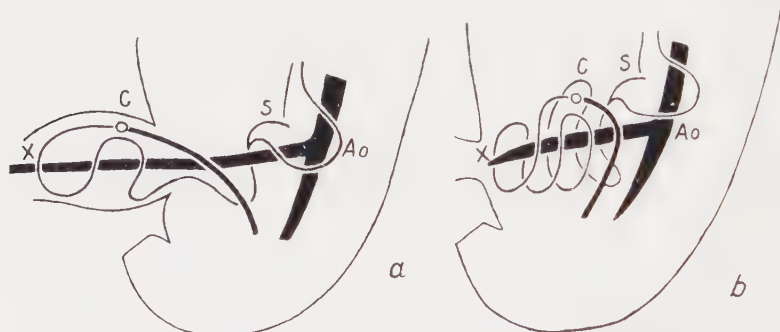


Fig. 1 a and b Diagrammatic side view of plastiline models of opossum embryos, (a) 9.4 mm. and (b) 11.2 mm. Linear course of the intestinal canal with the omphalomesenteric artery axial in the convolute system. In the older foetus the physiological umbilical hernia has been drawn in as a consequence of the vitelline artery having been shortened by one-half, the gastro-enteric coil in its entirety then being pressed together with dorsal displacement. *Ao* = aorta together with a. omphalomesenterica. *C* = coecum. *S* = stomach. *x* = point at which vitelline artery leaves mesentery. Large intestine drawn with thicker line. S. 1.

in their relations to the mesenteric portion of the vitelline artery, which portion forms the axis of the umbilical hernia.

The omphalomesenteric artery of the opossum is capable of a contraction of this kind with consequent replacement of the umbilical loops. At the time of the retraction of the extra-embryonic loops the vitelline artery is found to be equipped with a considerable longitudinal muscular apparatus. Hence the contraction of this artery can take place with the necessary power when an opportunity of shortening presents itself.

As a matter of fact this view is supported by an observation made by Rathke (1839). He found that the vitelline vessels in the common-snake embryo shorten towards the end of foetal development. No umbilical hernia has been observed in the foetus of the common snake, but the yolk sac wanders into the abdominal cavity. Respecting the cause of this Rathke writes (l.c., p. 184): "Was die Ursache der erwähnten Wanderung anbelangt, so liegt sie, wie ich glauben muss, in den Stämmen der Dottergefässe (Vene und Arterie), die sich gegen Ende des Fruchtlebens offenbar bedeutend verkürzen." The omphalomesenteric vein separates from the mesentery of the umbilical loop at an early stage and cannot therefore assist in the reduction of the umbilical hernia.

Especially great importance attaches to the vitelline circulation of the opossum as well as of marsupials in general, for during their short embryonic development no allantoic placenta is found. The vitelline circulation provides for all exchange with the maternal blood through the medium of a yolk-sac placenta and therefore attains to a comparatively high degree of development. As a retracting organ, however, only that part of the vitelline artery which is contained in the umbilical-loop mesentery can come into question. Now since this sooner or later always gives rise to an important intestinal artery, in man, e.g., the a. mesenterica cranialis, it cannot be regarded as improbable that this the axial arterial trunk of the umbilical hernia may also act in other cases at an opportune moment as a muscular retracting organ in the same manner as in the opossum.

The inquiry was therefore carried further with material, as before, from Prof. Ivar Broman's comparative embryological collections at the Tornblad Institute in Lund. And in the next work (Enbom, '38: 2) it was possible to show in embryos of *Homo* as well as of *Spermophilus citillus* and *Galeopithecus volans* a condition similar to that previously demonstrated in the opossum, viz. that on retraction of the umbilical hernia the axis of the umbilical loop is definitely shortened. Just as in this lowly mammal, so in the other mammals studied here, the reduction is accompanied by a dorsal displacement and a compression of the intestinal loops corresponding to the shortening of the vitelline artery that runs axially in the mesentery supporting the umbilical loop.

At this juncture the omphalomesenteric artery is the most powerful vascular trunk in the body, and its mesenteric portion is equipped with a longitudinal muscular apparatus of unusual strength. This impressive muscle, located axially in the system of intestinal convolutions, is manifestly to be regarded as a retracting organ of the best type to fulfill all requirements, and one that is not only able to re-

tract the umbilical loops but also to retain them within the abdominal cavity until the aperture in the anterior abdominal wall has had time to close.

Those cases in which the reduction takes place successively may be due to a differentiated tonicizing of the retracting muscle in the umbilical-loop mesentery, or to the resistance offered, when regression is delayed or stretching arrested, by the extra-mesenteric portion of the omphalomesenteric artery, the true *a. vitellina* (Broman, '14). On the other hand, should the longitudinal muscle of the omphalomesenteric artery be imperfectly developed, its activation be insufficient or arrested, a congenital umbilical hernia would be the consequence.

In the latest work (Enbom, '39) some further rules are advanced for the early looping of the alimentary canal in the mammalian and human foetus, and a discussion of the mechanisms that can be assumed to be active in this process is carried further. By way of introduction definitions are given of various types of loops (after Pernkopf, '25).

A basic plan is submitted of the primary looping. Four primary loops are defined: the gastric, duodeno-jejunal, umbilical, and colonic. The mechanism underlying the origin of the primary umbilical loop is discussed. It is shown that at the time it comes into existence the umbilical loop is regularly surrounded by two arterial trunks, the *aa. vitellinae*, with the result that the umbilical loop is directed between these arteries and grows out ventrally through the umbilical aperture. The twist of this loop from a sagittal to a transverse position appears, most probably, to be attributable to the strong growth of the liver, the umbilical loop being constrained to take up a transverse position with its limbs along the caudal surface of the liver, tangent to this.

The various coiling and shifting phases undergone by the gastric loop during its development are taken up for study. On the whole it appears probable that the stomach turns about a longitudinal, a transverse, and a sagittal axis. Respecting the two last-mentioned changes in the position of the primordium of the stomach, it is stressed that during the caudal migration of the gastro-intestinal system the pyloric portion becomes fixed in a position transversely across the omphalomesenteric artery.

A basic plan of the secondary looping is given. Three main groups of secondary loops arise in different planes around the omphalomesenteric artery: a sagittal group of dorsal loops, intra-abdominal loops of the small intestine to the right, a transverse group of ventral loops, extra-abdominal loops of the small intestine caudally, and to the left generally only a single sagittal loop of the colon, though in

some cases (e.g. *Spermophilus*, *Galeopithecus*) several secondary colonic loops form a separate sagittal group on this side of the artery.

This fact, that in certain cases both limbs of the primary umbilical loop are secondarily convoluted, suggests that it must be the vitelline artery which brings about the fixation of the top of the umbilical loop necessary for this convolution.

The same causes as bring the primary umbilical loop down into a transverse plane along the caudal surface of the liver, bring its extra-abdominal secondary loops to development in a transverse position caudally to this plane.

SUMMARY

Evidence has been submitted of the important part played by the omphalomesenteric artery in the development of the shape and position of the stomach and intestine in the mammalian and human foetus. This artery prevents the pyloric portion of the stomach from sinking caudally and therefore gives rise to characteristic changes in the position of the primordium of the stomach. It is a retractive organ for the umbilical loop, an organ that by arresting the latter's ventral growth first constrains the limbs of this loop to undergo secondary convolution and subsequently, on attaining full retractive power, brings about a drawing-in of the umbilical loops with a heaping together of the gastro-intestinal bundle as a whole—a force of the most manifest importance for the development of the gastro-intestinal system. The intestinal tube winds itself round the artery in question, forming typical groups of loops. In this way the omphalomesenteric artery comes to act as the principal axis of the intestinal bundle, and it is around this axis that the bundle turns.

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BOOK REVIEW

PHYSIOLOGY OF THE UTERUS, WITH CLINICAL CORRELATION. By SAMUEL R. M. REYNOLDS. Forewords by George W. Corner and Robert T. Frank. Paul B. Hoeber. New York and London, 1939.

In this book, "Dr. Reynolds has assembled and critically evaluated innumerable contributions scattered throughout the literature of anatomy, physiology, pharmacology and medicine, making them available for those interested" (from Foreword by Robert T. Frank). "For such a task Dr. Reynolds is particularly well prepared. Beginning his own studies, which themselves form a significant part of the total recent achievement in this field, in the physiological laboratory he has developed them as need required by drawing . . . upon the methods of embryology, biochemistry and the clinic" (from Foreword by George W. Corner).

For the first time there is available a synopsis of our knowledge to date of the physiology of the uterus. The anatomist will not look here for details of anatomy and histology, which are included only where the author has deemed a background of structure necessary, as in chapters VI and X which deal with the blood and lymph supply and the innervation of the uterus respectively. In the references cited, however, the interested student will find a good start for readings on the anatomical literature.

With regard to the endometrium, the author is quite right when he states (p. XIII) that "it is easy to obtain information pertaining to the structural changes which take place periodically in the endometrium." One might add that the physiological stimuli leading to these changes have also been frequently summarized. These are, however, not neglected in the present volume. The phenomena of menstruation are considered from a physiological standpoint, particularly with reference to vascular changes. These are dealt with, briefly but fairly, in the light of recent general reviews, of the work of Bartelmez and of Daron on the uterine blood vessels, and of the all-too-brief advance abstracts of Markee's direct observations on intraocular endometrial transplants. In the new edition, which is more than likely soon to be demanded of this extremely useful book, the reviewer would like to see a discussion of myometrial changes (vascular as well as tissue elements) associated with the menstrual flow.

The bulk of the book concerns the physiology of the myometrium. To the author is due the gratitude of biologist and clinician alike for presenting, in compact form, the enormous amount of scattered data on the subject. The author has, however, done more than that; for in a masterly fashion he has digested and classified the material and presented it in logical, natural sequence. Facts that would otherwise lack significance, he discusses in their broader physiological aspects, correlating, for example, known hormone responses with current physiological concepts, often pointing out principles common to the smooth muscle of all the viscera, including that of the uterus. The author is the more able to do this because he himself, on numerous fronts, has successfully attacked important phases of the subject in an outstanding manner.

After furnishing an orientation chapter on patterns of uterine motility, the modifying factors are successively discussed: hormonal control, in the pregnant as well as the nonpregnant state; growth (including the functional aspects of endometrial changes) as conditioned by both hormones and mechanical distention; changes in blood and lymph supply, according to the needs of the tissues; changes in metabolism, mineral content, pH, according to the reproductive phase. Three chapters deal with the pregnant uterus: V, Uterine Growth during Pregnancy; VII, Circulation and Respiration of the Gravid Uterus; XII, Physiological Relation in the Uterus at term—these three are of especial importance to obstetricians who wish to “render clinical procedures they employ less empirical.” The cause of birth is still not known, likewise the cause of menstruation; yet toward an understanding of both, as this book shows, many studies are being directed which are even now influencing medical practice.

The last chapter, XIII, on Physiological Basis of the Treatment of Disturbances of Uterine Muscle, written especially for the practicing gynecologist, is as optimistic as one can well expect a physiologist to be who respects control experiments as does the author. On the whole, the tenor of the book is not dogmatic—problems are not ‘settled,’ but rather hiatuses in our knowledge are pointed out. Thus chapter XI, on the Influence of the Uterus on Certain Bodily Functions, bristles with problems concerning which there exist immense amounts of contradictory data. Here the gynecologist will find a good review of the physiological effects of hysterectomy.

An excellent example of the author’s logical treatment of a topic is furnished by the section (in chapter IX) on the Knaus phenomenon and its use in the determination of the time of ovulation in women.

First the pitfalls of the method of recording contractions of the human uterus are detailed so that, when the merits of the Knaus theory are discussed, the reader is well able to follow the author in his evaluation of experimental data, both pro and con, bearing upon the theory.

In view of the obvious merits of the book both as to content and spirit, one should perhaps not criticize such a minor point as typography. However, the reviewer finds the author's method of making citations (compare author's justification on p. XIV) so time consuming and therefore irritating that he must enter his protest. Space is squandered by the method as well as by figures of billboard dimensions (e.g., 2, 15, 17, 25, 32). Glazed paper might easily have been avoided and both the glare of the page and the excessive weight of the book greatly reduced.

CARL G. HARTMAN,
Baltimore.

MACROSCOPIC STAINING OF THE BRAIN

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ONE FIGURE

INTRODUCTION

In 1926 Sincke introduced his Prussian blue method for staining macroscopic sections of the brain. Since then others have described various modifications and improvements in the original technique, and have brought us nearer, at the same time, to an understanding of the physio-chemical principles governing such reactions.

Two papers are of special interest. The work of Mulligan ('31) advanced the possibilities in this field, as with the introduction of carbolic acid into the staining process this author was able to improve markedly the clarity of his specimens. The results obtained by Blair, Davies and MacClelland ('32) disprove Sincke's suggestion that macroscopic brain staining has its foundation in the differences in iron content of gray and white matter. These authors were able to obtain good differentiation of the gray and white matter with various non-ferrous metallic salts, and even with the common starch-iodine reaction. Their results undoubtedly suggest that physical, rather than chemical, differences between gray and white matter dominate such reactions.

One obvious objection to most of these color reactions is that they look too artificial and too completely unlike what the student sees on dissecting the brain. Therefore, it was thought worth while to seek a reliable and comparatively simple color reaction which would mean only an exaggeration of the natural color differences between gray and white matter.

EXPERIMENTS

Sincke, and later Mainland ('28), drew attention to the fact that proper fixation was an essential factor in the staining process, but neither of them described the most satisfactory method, namely, perfusing the brain with the fixative.

In our experiments brains which were freshly obtained from the post mortem room were treated in the following manner. The Circle of Willis, after ligaturing the cut ends of the arteries, was slowly injected through a hypodermic needle with 10% formalin, particular attention being given to directing the fluid into the lenticulo-striate arteries. The vena magna cerebri was similarly injected. In some cases where too many blood vessels had been injured, thus obviating complete perfusion, the lateral ventricles were injected as well. Finally all the brains were immersed in an ample quantity of 10% formalin till fixation was complete. Injection in situ would be ideal but as our material was obtained from the post mortem rooms of hospitals we had to resort to the above methods.

Green ('33) suggested that secondary fixation in formalin, subsequent to sectioning the brain, greatly improved the quality of the staining. This we were able to confirm.

In our early experiments, free-hand sections of brain were used, but it was soon found that uniformity in the thickness of the slabs could not be obtained in this way. In order to get uniformly thick slabs cut in parallel series a simple 'cerebrotome' was designed with the aid of Professor Blair and Doctor Baesich. Such an instrument can be produced in any university workshop; the construction is illustrated in the accompanying diagram.

In our search for a satisfactory color reaction, the results of Blair, Davies and MacClelland formed the starting point. All the reactions described by them were repeated in their original form; afterward Mulligan's carbohic acid treatment was incorporated in the technique with notable improvement in the results. Various concentrations of carbohic acid were tried at various temperatures, but eventually it was decided

that the best results were obtained by the use of a 1 to 20 aqueous solution of carbolic acid at a temperature of 60 to 70°C. for 1 to 2 minutes.

It is a general complaint that the Prussian blue reaction fades in a few years time, but sulphide combinations are very stable and do not fade easily. The lead sulphide precipitation reaction described by Blair, Davies and MacClelland seemed

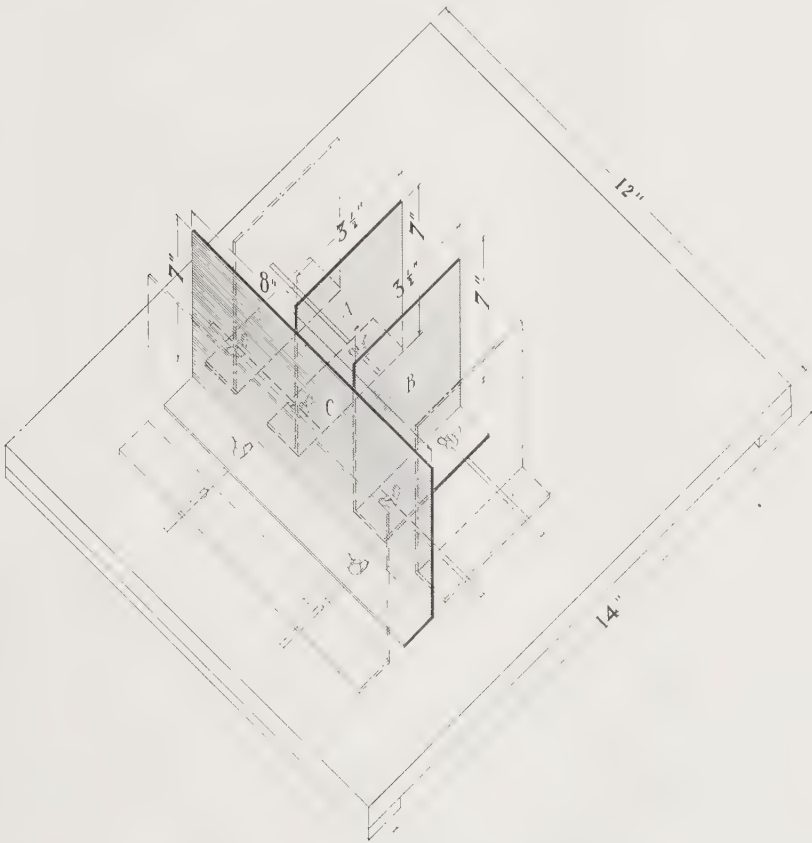


Fig. 1 A, B and C are stainless steel or chromium-plated brass plates, flanged at foot and clamped by thumb screws to the slotted hardwood base; the recessed slots on the under side of the base have brass linings. The brain is held between A and B, and cut face pressed against C, whose distance from A and B is adjusted for thickness of sections required. Knife is drawn through brain in contact with edges of A and B.

the most suitable for further experimentation because of the chocolate-brown staining of the gray matter and the stability of the sulphide combinations.

It was found that a mixture of 1% lead nitrate and 1% silver nitrate with subsequent immersion in ammonium sulphide gave the desired color rendering, the gray matter appearing in a color which was more or less an exaggeration of that of normal gray matter. A few drops of concentrated nitric acid were added to the nitrate solution to prevent the formation of a black precipitate which tends to occur. The addition of acid also improved the sharpness of the staining.

The final mounting of the finished specimens was planned so that they could be easily handled and examined by students, but at the same time protected from misuse. The well-known gelatine method was tried in glass 'boxes' made from opal glass backs, clear glass fronts, and glass strips for the sides, all cemented together. In these 'boxes' the stained sections were embedded in 10% gelatine.

However, difficulty was found in making the 'boxes' completely air tight with the result that evaporation and mold formation tended to occur, and finally we favored the method of mounting the specimens on glass sheets immersed in 10% formalin in the common type of sealed glass jars.

DISCUSSION

As already mentioned, fixation is of prime importance. If a fresh brain is fixed by simple immersion in a solution of formalin the outer parts in contact with the fixative become hard and prevent complete penetration of the fluid into the deeper parts of the brain, such as the basal ganglia, which thus do not become completely fixed and therefore do not assume the same physical texture as the external gray matter. This difficulty is got over by perfusing the brain with the fixative.

The action of the carbolic acid calls for some attention. On removing the brain slabs from the carbolic acid bath and afterwards immersing them in warm water (40 to 50°C.) for

5 to 10 minutes, the surface of the white matter appears to be covered with a jelly-like substance which is raised above the level of the adjacent gray matter. This jelly-like substance is a combination of myelin and carbolic acid. The myelin becomes partly dissolved while the slabs are in the carbolic acid bath and is precipitated on the surface of the white matter after washing out the carbolic in warm water.

Mainland noticed that if an area of the white matter is roughened by stippling with the bristles of a hard brush it becomes almost as deeply stained as the gray matter. After carbolic acid treatment such an area remains colorless because it becomes covered with the protective jelly. On the other hand, if a portion of the protective jelly is removed or damaged the site of the damage becomes deeply stained.

This shows that the protective jelly is important since it prevents the mineral salts from penetrating into the white substance and thus producing subsequent staining. Green noticed that after carbolic acid treatment the thalamic and hypothalamic regions did not stain so well. We have to remember that in these regions the gray matter is mixed with a large number of myelinated fibers, thus the more delicate staining is understandable. Nevertheless, by fixing the brain by the perfusion method the staining of these regions was always satisfactory.

That the carbolic acid by producing a protective jelly covering the white matter exerts a physical rather than a chemical influence in the staining process is further shown by the fact that its beneficial action seems to be a general one, equally improving the quality of the results after incorporating it in the various color reactions described by Blair et alia.

TECHNIQUE

1. Fix brain by perfusing 10% formalin through Circle of Willis and also through Vena Magna Cerebri. (If necessary the ventricles may also be injected.) Immerse in an ample quantity of 10% formalin till fixation is complete (10 to 14 days).

2. Wash brain in water to remove excess formalin.
3. Cut sections with cerebrotome.
4. Re-fix sections in 10% formalin for about 8 hours.
5. Wash in running water for 12 to 24 hours.
6. Immerse sections in 1 to 20 carbolic acid at 60 to 70°C. for 1 to 2 minutes.
7. Immerse in warm water (40 to 50°C.) for 5 to 10 minutes, so that protective covering becomes well developed. Do not touch upper surface of section during or after immersion in carbolic bath.
8. Harden protective jelly by immersion in cold water for a few minutes.
9. Immerse sections in solution of equal parts 1% lead nitrate and 1% silver nitrate (a few drops of concentrated nitric acid should be added immediately after mixing) for 1 minute.
10. Wash in running water for 10 to 15 minutes.
11. Immerse in ammonium sulphide 5 to 10% till good differentiation is obtained (about 15 seconds).
12. Wash in running water—protective jelly may now be removed by a strong current of water. If desired, the slabs may be mounted in 10% gelatine (add a few crystals of thymol to prevent mold, also a few drops of a 1% solution of crystal violet to counteract the yellowish tint of gelatine, together with egg white and egg shell, then clear by heating and filtering). Or, the slabs may be stitched to a sheet of glass in which holes are bored and then immersed in glass jars containing 10% formalin; the jars are then sealed.

I wish to express my thanks to Professor Blair and Doctor Bacsich for their continuous interest, helpful suggestions, and constant encouragement while producing this paper.

SUMMARY

1. A method of staining macroscopic brain sections is described that aims at an exaggeration of the normal color differences between gray and white matter.

2. The function of carbolic acid in improving macroscopic brain staining is discussed.

3. Illustration is given of a simple cerebrotome to facilitate the cutting of thick brain sections.

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SEROSAL AND MUCOSAL DIMENSIONS AT DIFFERENT LEVELS OF THE DOG'S SMALL INTESTINE

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THREE FIGURES

The irregularity of the mucosal surface of the small intestine produced by the configuration of the villi and of the valvulae conniventes (folds of Kerkring) has an obvious functional significance. It makes available a large epithelial area for the rapid absorption of nutrient material from the intestinal lumen. The need for a numerical expression of the proportional amounts of such areas at different levels of the intestine makes itself felt to the anatomist, who wishes to describe accurately the intestinal tract as a whole, to the surgeon, who wishes to know the amount of functional absorbing surface he removes when he resects a given length of bowel, and particularly to the physiologist, who, in his studies on the absorption velocities of various materials, wishes to know the surface areas of his experimental loops.

A summary of the significant previous work on the subject is given in table 1. In reviewing this work several facts of interest stand out. As nearly as can be determined in each case, the calculations depended upon measurements of individual villi either in mounted specimens of fixed intestine or in the living animal by the use of the ocular micrometer. The areas of individual villi were calculated arbitrarily from their linear dimensions, assuming them to be cylindrical in

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shape. The number of villi per unit area were then counted and the mucosal area thus approximated. There is considerable disagreement in results, most notably between those of Heidenhain (1888) and of Krogh ('29) on the dog. This is due to the fact that Spee (1885), from whose work Heidenhain made his calculations, procured average lengths for villi about double

TABLE 1
Literature on intestinal surface area

AUTHOR	ANIMAL	METHOD	INTESTINAL LEVEL	MEASUREMENTS
Heidenhain (1888) (from Spee, 1885)	Dog	Fixed specimens (osmic acid)	Not specified	Villus surface = 0.96 mm. ² 23 cm. ² for each cm. ² surface
Mall (1887)	Dog	Fixed specimens	Not specified	Submucosal surface 657 cm. ²
Krause (1890)	Man	Fixed specimens	Whole small intestine	Villus surface = 0.3 to 0.7 m. ² 4,000,000 villi in small intestine = 1.6 sq. meters. Multiplication of area is 5 ×
Krogh ('22) (from Mall, 1887)	Dog	Fixed specimens and calculations from Mall's data	Not specified	7 mm. ² for each 1 mm. ² surface
Vimtrup (quoted by Krogh, '22)	Rabbit	Fixed specimens	Duodenum	17.6 mm. ² for each 1 mm. ² surface
Verzàr ('36)	Pigeon	Direct measurements in the living	Small intestine	Total surface 689 cm. ² . Ratio of mucosal to serosal area is 8/1, 3rd of it in duodenum
Maximow and Bloom ('38)	Man	Not stated	Small intestine	Mucosal surface = 4 to 5 sq. meters

those obtained by Mall (1887) from whom Krogh got his data. Review of their work does not suggest that the discrepancy arose from using different and non-comparable levels of the intestine. None of the workers, except Verzàr and McDougall ('36) has emphasized difference in mucosal area per unit of length at different levels, and their work was on the pigeon.

All authors engaged in this work, including the present one, have recognized that any value used to express the whole mucosal surface area of the intestine or the area per unit length can be only a crude approximation. This is so not only because in the living animal the intestine is continually changing length as the tonus of its longitudinal muscle varies (van der Reis and Schembra, '26), but also because, even if the length were constant, the individual villi in most species are continually changing their shape by pumping movements (King and Arnold, '21; Verzàr and McDougall, '36; Spee, 1885) with resultant change in surface area (Maximow and Bloom, '38; Heidenhain, 1888). Estimated areas are used in this work to show tendencies and trends because of the unavailability of exact measurements. Because of the sources of error inherent in the method this work is presented not as a final solution to the problem, but as adding some interesting data to those already accumulated. The method, which makes use of total linear dimensions rather than measurements of individual villi, is different from any heretofore used. Especial attention is paid to variations in mucosal surface at different intestinal levels.

PROCEDURE

After prolonged but fruitless attempts to procure a human intestinal tract early enough post mortem to secure fixation before autolysis of the epithelium had occurred, the small intestine of a 12 kg. dog was chosen for study. The dog, which the day before had had a laparotomy performed for other purposes, was sacrificed by intracardiac injection of 20 cc. of choroform. The whole small intestine was immediately removed, closely shorn of its mesentery, and glass cannulas were tied into each end. Formalin, 10% solution, was injected into the cannula at the duodenal end under a pressure of 60 cm. of water. A large part of the intestinal contents were thus washed out of the lower cannula. After 5 minutes of washing the lower cannula was closed and the whole specimen allowed to distend under the 60 cm. pressure of the

formalin. When the distention appeared to have reached its limit the injection was stopped and the upper cannula was closed leaving the contents of the specimen under pressure. The intestine was then laid in two 10 foot troughs full of formalin. After fixation, lengths, approximately 8 cm. long, were cut from the duodenum, mid jejunum, upper, mid and lower ileum. Each of these lengths was in turn cut into three pieces (fig. 1), a center length of 5 cm., and two end lengths of about 1.5 cm. each. After washing, hardening in alcohol, and embedding in celloidin, transverse sections were cut from each of the end pieces and longitudinal sections from the

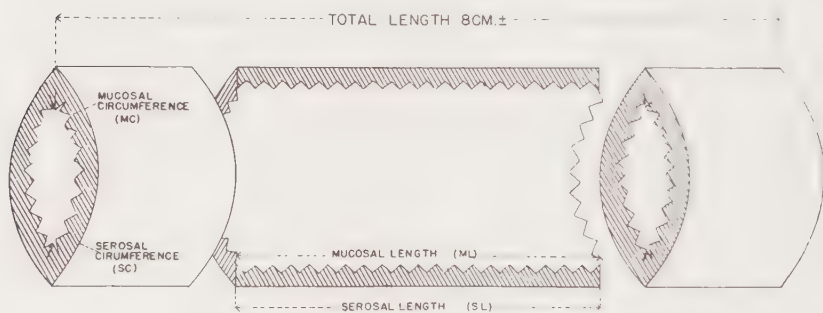


Fig. 1 Diagrammatic representation of the manner in which the segments were cut up.

center pieces. These were placed on slides, stained with hematoxylin and eosin, and mounted in balsam.

The slides were projected with a Bausch and Lomb projecting drawing apparatus onto a screen where the image was magnified eighty times. The serosal and mucosal outlines as shown on the screen were measured in their circumferential dimensions on the transverse sections, and in their longitudinal dimensions on the longitudinal sections with a rotometer, or measuring wheel. The average thickness of the tunica muscularis was recorded. By taking the average of circumferential values procured for the two end lengths and the average of the longitudinal values for the two sides of the

center length, ratios of mucosal to serosal circumference and of mucosal to serosal length were calculated for each segment.

No exact formula for computation of the area of an irregular surface of undefined shape in terms of two dimensions

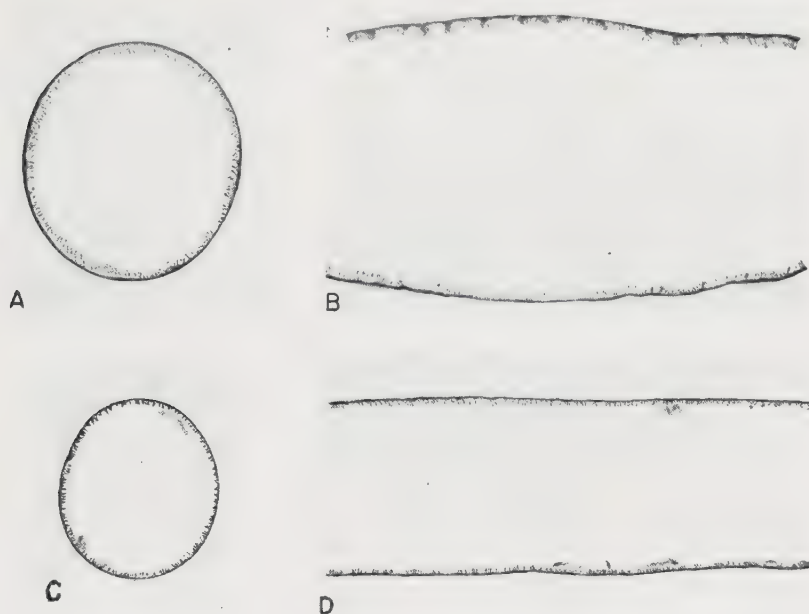


Fig. 2 A, B, C, and D, low power ($\times 1.8$) magnifications of transverse and longitudinal sections from the duodenum (A and B) and the ileum (C and D). For high power magnification of B and D, see figure 3.

can be derived. The following analysis was kindly prepared for us, however, by Dr. J. H. Austin.

If SC = Serosal circumference
 MC = Mucosal circumference
 SL = Serosal length
 ML = Mucosal length
 SA = Serosal area
 MA = Mucosal area

Then in general for an irregular surface

$$\frac{MC}{SC} \text{ or } \frac{ML}{SL} \text{ (whichever is greater)} < \frac{MA}{SA} < \left(\frac{MC}{SC} \times \frac{ML}{SL} \right)$$

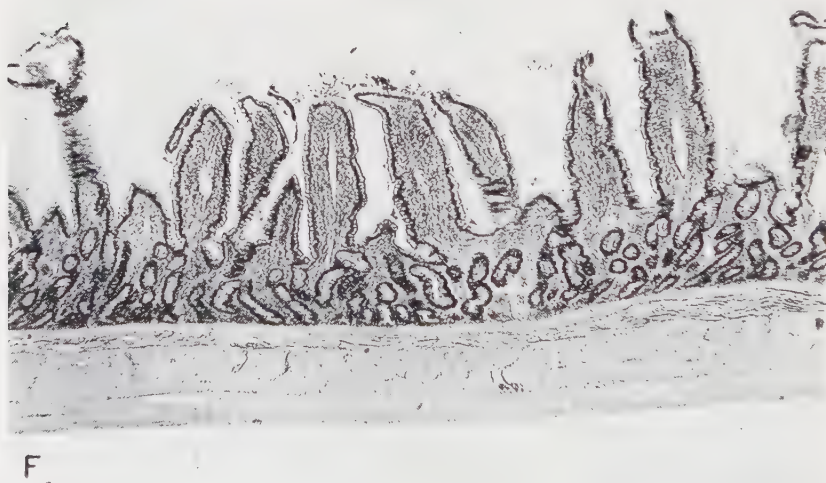
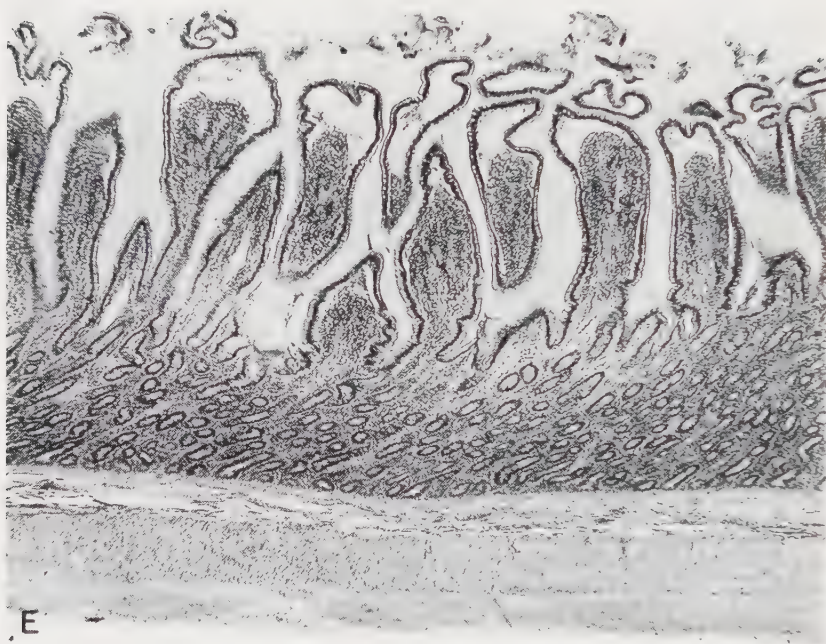


Fig. 3 E and F, high power ($\times 50$) magnifications from the same levels as shown, respectively, at B and D, in figure 2.

TABLE 2
Measurements and calculations—dog's small intestine

LEVEL OF SEGMENT	DISTANCE FROM PYLORUS*	AVERAGE MC	AVERAGE SC	AVERAGE ML	AVERAGE SL	AVERAGE THICKNESS MUSCULARIS	MC/SC	ML/SL	EA/SA	EA PER CM. OF SL	TOTAL SL†	TOTAL SA†	TOTAL EA†
	cm.	cm.	cm.	cm.	cm.	mm.				cm. ²	cm.	cm. ²	cm. ²
Duodenum	5	41.4	7.6	41.0	4.64	0.34	5.43	8.84	13.27	101	12	91	0.12
Jejunum	91.2	26.5	6.4	27.06	5.05	0.23	4.14	5.36	8.50	54	128	820	0.69
Upper ileum	169	25.35	6.35	22.8	5.03	0.25	3.99	4.53	7.52	48	60	381	0.29
Mid ileum	230	18.73	5.7	22.2	4.93	0.23	3.28	4.59	6.78	39	60	342	0.23
Lower ileum	289	16.8	3.95	34.4	4.85	0.38	4.25	7.09	10.34	41	70	276	0.29
Whole small intestine													
									8.5	49	330	1910	1.62

* Upper end of segment.

† The last three columns give values for the whole part of the intestine that each segment is taken to represent. They are important as essential steps in the calculation of the values for the whole small intestine which appear at the bottom of those columns.

Examination of various measurable geometric projections indicates that a serviceable approximation for MA is an estimated area, EA, calculated with the following formula

$$\frac{EA}{SA} = \frac{MC}{SC} + \frac{ML}{SL} - 1$$

It is not possible to state the deviations in any surface of unknown shape between EA and MA. We have, however, calculated EA from our data as a plausible approximation to the mucosal area.

RESULTS

In table 2 the figures to the left of the double line are accurate measurements and calculations. It can be seen that the ratios MC/SC and ML/SL decrease as the lower parts of the intestine are reached with the notable exception of the lowest specimen of all. This shows an increase to figures lower only than the duodenal values. The explanation for this is obvious if one examines the values for circumference and thickness of muscle layers in this segment. They indicate, as was apparent on inspection of the specimen, that in the last 75 cm. the ileum was in spasm not overcome by distention with the fixative. The values for this segment are, then, not comparable with those of other segments where distention was obtained with the fixative.

The figures to the right of the double line in table 2 represent values calculated from the approximation formula. A progressive decrease in estimated mucosal area per centimeter of serosal length is observed as one passes downward to the mid ileum. The estimated mucosal area for the duodenum, or 0.12 m.², comprises about 7% of that estimated for the whole small intestine.

DISCUSSION

Sources of error inherent in the technique itself have been examined. Numerous measurements before and after fixation were made and support the observations of Patten and Philpott on chick embryos that no shrinkage takes place in 10%

formalin. Contrary to their results, however, no shrinkage in the serosal dimensions was found in this work between fixation and mounting. The villi were not susceptible of measurement from this point of view. Probably shrinkage occurred. Patten and Philpott ('20) estimated that using paraffin embedding the maximum shrinkage with this technique was about 20%. The larger the specimens, the smaller the

TABLE 3
Duplicate measurements on same mucosal dimensions

LEVEL	MAGNIFICATION	DIMENSION	FIGURES	DIFFERENCE
			<i>cm.</i>	<i>%</i>
Duodenum	× 19	MC	31.9-35.3	10.1
Lower jejunum	× 80	ML	22.7-22.8	0.4
Upper ileum	× 80	ML	21.9-23.4	6.6
Upper ileum	× 80	ML	21.9-21.9	0.0
Upper ileum	× 80	MC	19.3-19.7	2.1

TABLE 4
Measurements of two sections from similar levels

LEVEL	MAGNIFICATION	DISTANCE APART	DIMENSIONS COMPARED	FIGURES	DIFFERENCE
				<i>cm.</i>	<i>%</i>
Upper jejunum	× 80	100 μ	MC	26.4-27.9	5.5
Upper jejunum	× 80	100 μ	SC	6.6- 6.5	1.5
Upper jejunum	× 80	Opposite sides	ML	26.6-27.5	3.3
Upper jejunum	× 80	Opposite sides	SL	5.1- 5.0	2.0
Upper jejunum	× 80	100 μ	ML	26.6-27.5	3.3
Upper jejunum	× 80	100 μ	SL	5.1- 5.0	2.0
Upper ileum	× 19	Opposite sides	ML	18.0-17.7	1.7
Upper ileum	× 19	Opposite sides	SL	5.0- 5.0	0
Mid ileum	× 80	Opposite sides	ML	21.9-21.9	0
Mid ileum	× 80	Opposite sides	SL	4.9- 5.0	2.0

percentile shrinkage. Since celloidin embedding was used here, it is probable that the amount of error from this source was less than 10%. Table 3 gives data on re-measurement of the same mucosal dimensions with the rotometer. Table 4 shows measurements of similar but not identical sections within 100 to 300 μ of each other, or, in the case of the longitudinal sections, from the opposite wall, to determine how

representative is the measurement of a single section. The average difference was 3.3% for mucosal measurements and 1.5% for serosal.

These results are applicable only to the intestine of the dog. This animal has a much less abrupt change in diameter between the duodenum and the rest of the small intestine than the human. It has, moreover, no valvulae conniventes that are evident in preparations of this sort, although functional transverse folds are the rule in the living. The valvulae in the human intestine cannot be flattened out by distention of the lumen (Maximow and Bloom, '38). It is obvious that MA/SA diminishes in man also, the lower the segment of intestine considered; it is probable, however, that the ratio of mucosal to serosal surface area is greater in man than in the dog.

Van der Reis and Schembra ('26) measured the percentile increase in length along the mesenteric border of the dog's small intestine at various intervals after the death of the dog and also after removal of the mesenteric attachments. If one applies their results to this intestine an approximate increase in serosal length of from 60 to 90% above the living values may be assumed to have occurred. Rough estimations of the mucosal area per unit length in the living dog may be arrived at by taking this into consideration.

Comparing our average value for EA/SA, 8.5, with the values reported for the dog in table 1 it can be seen that our value is much lower than that of Heidenhain (1888), and approximates that of Krogh ('29).

It seems to the author that the technique here used would be of value applied to human intestine.

SUMMARY

1. The mucosal and serosal linear dimensions, both longitudinal and circumferential, at five levels of the autopsied small intestine of a dog have been measured and the error of these measurements has been indicated.

2. A formula is suggested which from these dimensions permits a crude estimate of mucosal area.

3. The estimate of the ratio of mucosal area to serosal area for the whole small intestine was 8.5/1.

4. The estimate of the total mucosal surface was 1.6 square meters of which 7% was in the duodenum.

5. Striking decrease in mucosal area per unit of serosal area or of serosal length is observed as one descends the gut to the mid ileum.

6. The impropriety of applying these results directly to the human subject is emphasized.

In addition to the invaluable advice of Dr. J. H. Austin, I wish to express my appreciation of the assistance given me by Dr. Balduin Lucké, who helped to devise the method, and by Mr. Basil B. Varian, who prepared the sections.

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ON THE FUCHSINOPHILE AND PALE CELLS IN THE ADRENAL CORTEX TISSUE OF THE FOWL

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ONE PLATE (THREE FIGURES)

In an earlier paper (Uotila, '39) it was noted that in fowls the adrenal cortical tissue was made up of cells with two distinct staining reactions—the so-called fuchsinophile cells on the one hand, and the pale cells on the other. These could be clearly distinguished after Orth's fixation and the Masson trichrome stain. In this paper a further cytological description of these cells will be presented, together with observations on the changes in these cell types under various natural and experimental conditions such as age and sex differences and changes produced by stimulation or depression by various hormones.

In addition to the well-established function of the adrenal cortex connected with sodium metabolism, this tissue has been stated to have some relationship to the production of male hormone. This latter function has also been suspected in birds (Zawadowsky, '22; Riddle, '25; McGowan, '36). The following studies were designated to find out whether the fuchsinophile or pale cells are specifically concerned with either of these two alleged adrenal cortical functions. That such a relationship might exist was suggested by the observations of Broster and Vines ('33) who noted that in cases of human adrenal virilism, there was a strong fuchsinophile reaction in the cortical tumor tissue. This fuchsinophile reaction was noted to be absent in normal human and mouse adrenals.

¹ Holder of a Finnish Government Fellowship (1938). This work has been partially aided by a grant from the Josiah Macy, Jr. Foundation.

MATERIAL AND METHODS

Ninety-nine pullets, cockerels, roosters, and hens were used as experimental and control animals. The exact number and category of birds used in each experimental procedure will be found under 'Observations.'

Sex and age differences were studied in adult hens and roosters and in 24-day-old pullets and cockerels. Anterior pituitary extract (Squibb), corticotropic extract (Collip), and FSH and LH (Fevold) were injected into pullets in attempts to stimulate the adrenal cortical tissue. In cockerels and pullets the effects of dihydro-androsterone benzoate (Oreton B, Schering) and estradiol benzoate (Progynon B, Schering) were noted.² All injections were made subcutaneously in the pectoral region, and started when the birds were 4 days old.

The directions given by Broster and Vines ('33) for the stain they used for demonstrating fuchsinophile tissue in human adrenals is as follows: after formol-dichromate fixation,

1. Stain with ponceau fuchsin, 3 minutes.
2. Wash in aq. dest.
3. Differentiate in 1% aq. solution phosphomolybdic acid.
4. Stain 5 minutes in anilin blue, sat. sol. in 2.5% acetic acid.
5. Wash in aq. dest.
6. Differentiate in 1% acetic acid.
7. Wash, dehydrate, and mount in salicylate balsam.

It will be noted that Broster and Vines fail to give precise directions for making up the ponceau fuchsin stain. In view of this fact, a 1% solution of Grübler's ponceau fuchsin in 1% acetic acid was tried and was found to give a characteristic fuchsinophile stain to certain adrenal cortical cells in fowls after Orth's fixation. It was then found that the Masson III

²The author is grateful to Dr. J. B. Collip, Department of Biochemistry of McGill University, to Dr. H. L. Fevold, Biological Laboratories of Harvard University, to Squibb Sons, New York City (courtesy of Dr. H. S. Newcomer), and to the Schering Corporation of Bloomfield, New Jersey (courtesy of Dr. M. Gilbert), for their generosity in supplying him with the hormones used in this study.

trichrome stain described by Foot ('33), using Regaud's iron hematoxylin and light green instead of anilin blue as a counterstain differentiated the fuchsinophile and pale cells of chicken adrenals fully as well as the Broster-Vines stain, and in addition gave far superior nuclear and cytoplasmic detail. It was found advantageous to use thin (4-5 μ) sections and 30 minutes differentiation with phosphomolybdic acid. Chondriosomes were stained with the Altmann-Kull's aniline acid-fuchsin crystal violet method after Orth's fixation. Some preparations were stained with Heidenhain's iron-hematoxylin, and alum-hematoxylin and eosin.

OBSERVATIONS

1. *General features of cortical tissue in fowl.* The arrangement of the cortical and medullary tissue in fowl differs to some extent from that found in mammals. The cortical tissue appears as irregular cords between which the medullary tissue interdigitates intimately throughout the whole gland. Consequently no distinct cortical zoning is present, as is the case in mammals (figs. 1 and 2). This cortical tissue contains both fuchsinophile and pale cells.

The pale cells stain light green with the Masson trichrome method and are usually roundish or polygonal in cross section or wedge-shaped in longitudinal section. The cytoplasm shows a large or small number of vacuoles of varying size which presumably contain lipin before dehydration. Between the lipin vacuoles one can sometimes see scattered fuchsinophile granules. The nucleus is round, stains lightly, and shows two or three nucleoli (fig. 3). The chondriosomes are fewer and stain more feebly than in the fuchsinophile cells, and are granular or vacuolated (mitochondria).

The fuchsinophile cells present two aspects: one showing definite degenerative changes; and the other, none. Those fuchsinophile cells which are not degenerating resemble pale cells except that their cytoplasm contains, besides the lipin vacuoles, numerous fuchsinophile granules many of which are vacuolated. This granulation is often obscured by the

diffusely staining cytoplasm (fig. 3). The Altmann-Kull stain reveals numerous granular or vacuolated mitochondria which are most abundant in the capillary pole of the cells. Evidently much of the fuchsinophile staining in these cells shown by the Masson stain after Orth's fixation can be ascribed to mitochondria.

Those fuchsinophile cells which show degenerative changes are often compressed into a stellate form or pushed to the periphery of the cortical cords. The cytoplasm takes a deep, diffuse fuchsin stain and no granules are visible (fig. 3). These cells stain darkly with iron hematoxylin (siderophilia), or crystal violet, and exhibit increased pigmentation and pycnotic nuclei. Some of the degenerating cells possess irregular or vacuolated chondriosomes.

The fuchsinophile reaction is absent after Zenker fixation, although even then, the fuchsinophile cells can be distinguished and show numerous, non-specific, grayish-brown granules.

2. *The influence of age and sex.* Five adult roosters, five adult hens, twenty cockerels, 24 days old, and twenty pullets of the same age were used for these observations.

Although accurate quantitative estimations of the percentage of fuchsinophile cells were attempted, this was difficult to achieve because of the great variations in different parts of the gland and in various individuals. In general it seemed that in 24-day-old cockerels, 75-90% of the cortical cells were fuchsinophilic (fig. 1), whereas in pullets of the same age only 40-60% exhibited fuchsinophilia (fig. 2). The cortical cells of adult roosters were about 60-70% fuchsinophilic, whereas in adult hens only 30-50% showed fuchsinophilia.

3. *The effect of whole anterior pituitary extract.* Seven pullets were injected with 0.5 cc. of APE every day for 20 days, and compared with five controls. The weights of the adrenals in the injected birds were increased (26.6 mg. vs. 20.4 mg. in controls), but there were no definite changes in the cytology or percentage of the fuchsinophile cells.

4. *The effect of corticotropic extract.* Five pullets were injected with 0.4 cc. of corticotropic extract daily for 20 days and compared with seven uninjected controls. No changes were produced in the weight (25.5 mg. vs. 26.0 mg. in controls), cytology, or percentage of fuchsinophile cells. Subsequent tests showed the extract to be potent in rats.

5. *The effect of FSH.* Five pullets received 5–10 units of FSH daily for 20 days, and were compared with seven controls.

The results were again negative as regards the weights (22.0 mg. vs. 26.0 mg. in controls) and the cytology of the adrenals. This treatment produced marked increase in the weights of the ovaries, oviducts, and thyroid, as well as masculinization of head furnishings (Uotila, '39).

6. *The effect of LH.* Five daily units of LH were given to four pullets for 20 days while seven birds served as controls. This treatment failed to cause changes in the weights (25.0 mg. vs. 26.0 mg. in controls) or the cytology of the adrenals. The weights of the thyroids and ovaries were increased by these injections, and head furnishings were masculinized. The weights of the oviducts were not changed significantly (Uotila, '39).

7. *The effect of dihydro-androsterone benzoate.*

a. Seven pullets were injected with 0.5 mg. of androsterone every other day for 20 days and compared with five controls. No changes were observed in the weight (21.8 mg. vs. 20.4 mg. in controls), in the cytology or in the percentage of fuchsinophile cells in the cortex.

b. Six cockerels were given 1 mg. of androsterone every other day for 20 days. Nine cockerels served as controls. After this treatment the adrenals showed some atrophy (26.3 mg. vs. 32.0 mg. in controls) with a definite decrease in the percentage of fuchsinophile cells.

8. *The effect of estradiol benzoate.*

a. Seven pullets were treated with 500 I.U. of estrin and the results compared with five controls. No clear changes

appeared either in the weight (23.0 mg. vs. 20.4 mg. in controls) or in the microscopic appearance of the adrenals.

b. Five cockerels each received 500–750 I.U. of estrin every other day for 20 days. Nine birds were kept as controls. The weights of the adrenals in the injected birds averaged 36.5 mg. as compared with 32 mg. in controls. The percentage of fuchsinophile cells was somewhat decreased.

DISCUSSION

The above observations raise the question as to whether the fuchsinophile and pale cells respectively are in reality distinct cell types or represent merely different phases of a single cell type.

The cytological appearances in our preparations can be best interpreted in terms of the latter possibility. Perhaps the strongest evidence in favor of a single cell being involved is the fact that in every specimen one can find numerous transitional stages from pale to fuchsinophile cells. One gains the impression that the pale cells may represent younger stages which, in the course of cytomorphosis, are transformed into fuchsinophile cells which, in turn, finally become senescent and constitute the degenerating type of fuchsinophile cells.

It has been noted repeatedly by several workers that in the thyroid increased activity is accompanied by augmented size and numbers of chondriosomes (Uotila, '34). If the number and size of the chondriosomes in the adrenal cortical cells can be considered as indicative of cell activity, the non-degenerating fuchsinophile cells of the fowl with their numerous chondriosomes, should be very active, whereas the pale cells in which the chondriosomes are scanty and lightly staining, should be less active.

Besides the cells which present evidence of activity, some of the fuchsinophile cells show unmistakable signs of degeneration, with pyknotic nuclei, darkly staining compressed cytoplasm, increased pigmentation, diffusely massed chondriosomes, and compressed or distorted cell shapes. The affinity of these cells for iron hematoxylin and crystal violet resembles

similar staining reactions of the cells in the inner layers of the mammalian adrenal cortex. These degenerating fuchsinophile cells often appear pushed and compressed toward the periphery of the cortical cords, whereas the pale cells are most abundant in the central parts of the cords. This observation suggests that as the pale cells mature and become fuchsinophilic and later degenerate, they tend to be forced from the centers of the cords toward the periphery in a manner somewhat similar to the apparent movement of cells in nodules of cortical tissue embedded in the medulla of mammalian adrenals (Bennett, unpublished data).

The available data indicate that the relative number of fuchsinophile cells is greater and the staining reaction more intense in male than in female birds and in immature than in adults. It is also noted that androsterone, and to a lesser extent, estrin, decrease the fuchsinophile reaction in males but not in females. None of the anterior pituitary extracts tried in these experiments caused any noticeable change in the fuchsinophilia.

These results suggest that the fuchsinophile reaction in chickens is somewhat influenced by sex, age, and certain sex sterols. In this respect it resembles to a certain extent, the X-zone of mice. It cannot be said whether these influences specifically affect the cytomorphosis of the adrenal cortical cells, or whether the changes noted are caused by other metabolic factors.

According to Broster and Vines ('33) the fuchsinophile reaction is present in man in masculinizing cortical tumors, in the normal cortical tissue of human embryos of both sexes at certain stages, in the eosinophilic cells of the anterior pituitary, and sometimes in the interstitial cells of the testis and in the peripheral cells of young corpora lutea. Using Broster and Vines' method, or the Masson stain, we found fuchsinophile material in sympathetic ganglion cells, in the smooth muscle of the walls of arteries, besides in many other cells. Therefore, as Broster and Vines have, themselves, recognized, and in spite of the fact that fuchsinophilia in the

human adrenal cortex is associated with masculinization, the cytoplasmic constituents involved in the reaction can scarcely be considered as a source for any specific substance. The fuchsinophile material has staining properties somewhat similar to those of chondriosomes. It is preserved by Orth's fixation and destroyed by Zenker fixation, shows a strong affinity for iron hematoxylin, crystal violet and eosin. Hence the fuchsin-staining material in the cells also resembles to a certain extent the so-called 'siderophile substance' in the inner zones of mammalian adrenals, the nature of which is entirely unknown.

Bourne ('35) has observed similar staining properties of the fuchsinophile material and chondriosomes in one case of adrenal virilism. From this observation he has advanced the theory that in cases of adrenal virilism the increased number of chondriosomes acts catalytically in transforming adrenal sterols into male hormone. This concept has no direct, supporting proof.

In contrast to normal human adrenals, those of fowl display the fuchsinophile reaction as a regular feature. This reaction in the chicken adrenal is somewhat influenced by sex, age, and sex sterols. Nevertheless, the present material affords no definite proof that fuchsinophilia is specifically correlated with masculinizing function or substance, as postulated by Broster and Vines for the human, even though the studies of Zawadowsky ('22), Riddle ('25), and McGowan ('36) suggest the presence of an androgenic substance in the adrenals of fowl.

The author wishes to express his deep gratitude to Dr. George B. Wislocki for his interest and kindness during the work, and to Dr. H. S. Bennett who corrected the English.

SUMMARY

Two cytomorphic states—the fuchsinophile and pale cell respectively, can be regularly and clearly differentiated in the adrenal cortical tissue of the fowl when stained with Broster and Vines' ponceau-fuchsin or Masson trichrome stain after Orth's fixation.

Cytological criteria suggest that the pale cells are precursors of active fuchsinophile cells which in turn become converted into degenerating fuchsinophile cells.

The number of fuchsinophile cells and the strength of the fuchsinophile reaction is greater in males than in females and greater in immature than in adult animals. Androsterone and (to a lesser extent) estrin suppress this reaction in males but not in females. Several anterior pituitary extracts failed to produce any definite changes in the fuchsinophile reaction.

The material colored by fuchsin has staining properties in many respects similar to those of the so-called 'siderophile substance' and to chondriosomes.

The present observations afford no definite proof that the fuchsinophile reaction is specifically correlated with the alleged masculinizing hormone or function of the adrenal cortex.

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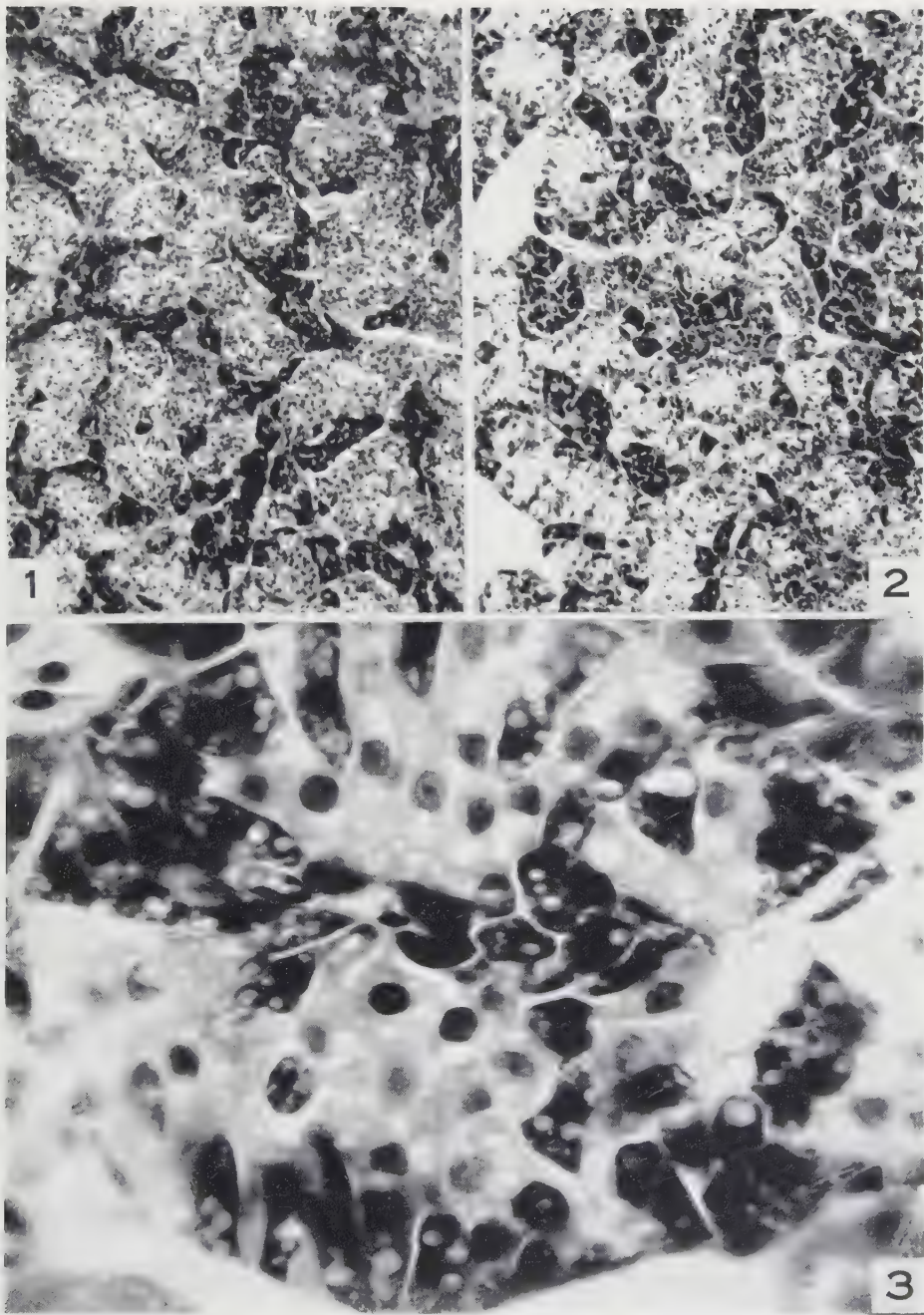
PLATE 1

EXPLANATION OF FIGURES

1 Adrenal gland of 24-day-old cockerel. Note the irregular cortical cords between which the dark medullary tissue intervenes. In the cortical cords fuchsinophile cells preponderate with only a few pale cells. Orth's fixation, Masson trichrome. $\times 160$.

2 Adrenal gland of 24-day-old pullet. The fuchsinophile cells are not as numerous as in figure 1. Orth's fixation, Masson trichrome stain. $\times 160$.

3 Adrenal of 24-day-old pullet. Note the differences between the pale and fuchsinophile cells. The pale cells are most abundant in the middle of the cortical cord, whereas fuchsinophile cells are more plentiful at the periphery. Some of the fuchsinophile cells are undergoing degeneration, more especially the cluster of dark cells visible in the upper left hand corner. Orth's fixation, Masson trichrome stain. $\times 1050$.



RELATION OF THE VOLUME OF PULMONARY CIRCULATION TO RESPIRATION AT BIRTH

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It is commonly taught that very little blood courses through the atelectic fetal lungs but that with the advent of air breathing and the discarding of the umbilical circuit at birth, a re-routing of blood into expanding pulmonary channels takes place with dramatic suddenness. This conception has been questioned by Patten and his colleagues who have presented impressive anatomical evidence that the change in pulmonary circulation is gradual (Patten, Sommerfield and Paff, '29; Patten, '31, '33).

The orifice of the foramen ovale is so restricted by attachments of its flap-like valve in late fetal life that it alone cannot be assumed to deliver enough blood to the left atrium to bring about an equalization of pressure there with that in the right atrium. And yet it has been proved that the two ventricular pressures are practically equal in the fetus before breathing starts (Pohlman, '09; Hamilton, Woodbury and Woods, '37). Pulmonary venous return would seem to make up the difference and thus actually to supply more blood to the left atrium than does the functional orifice of the foramen ovale. However, there are few data, other than Patten's measurements of vessel diameters supporting this view. Indeed, most of the other experimental evidence seems to be adverse.

Both Pohlman ('09) and Kellogg ('28) found that when a suspension of starch was introduced into the superior or inferior vena cava of a living apneic fetus equal amounts

¹ Doctor Windle, aided by a grant from Child Neurology Research (Friedsam Foundation).

could be recovered from the two ventricles. If there were a significant volume of blood entering the left side of the heart from the fetal lungs and diluting the starch laden blood from the foramen ovale, these investigators should have recovered more starch grains from the right ventricle than from the left.

The systolic carotid arterial pressure increases greatly with the advent of breathing at birth in the sheep (Barcroft, '38). However, the pulmonary arterial pressure may be no greater after birth than it was in late fetal life. The immediate effect of respiration in the rabbit and dog has been reported (Hamilton, Woodbury and Woods, '37) to be a reduction of both intra-ventricular pressures with the right side much more affected than the left. This seems to be due to establishment of negative intrathoracic pressure. It has been suggested that a relatively greater filling pressure of the left ventricle follows the shift to air breathing and results in almost immediate functional closure of the valve of the foramen ovale. One naturally suspects too that an actual increase in volume of the venous return from the lungs plays a part in raising the filling pressure but there is no proof of this in the experiments which have been performed.

In view of the diverse and apparently contradictory lines of evidence concerning the status of the fetal pulmonary circulation and the changes therein concomitant with birth, we have endeavored to obtain some sort of quantitative estimation of the volume of blood within this circuit both in fetuses which breathed no air and in their litter mates which were allowed to breathe. A comparison of such should reveal any immediate alterations in pulmonary volume brought about by the initiation of respiration.

MATERIALS AND METHODS

With the exception of a single guinea pig, our experiments were performed on cats in which pregnancy was terminated experimentally 50 to 67 days after insemination. We operated upon nine pregnant cats obtaining thirty-three fetuses, five

of which were employed for purposes irrelative to this experiment, one of which was discarded because of a large umbilical hernia and twenty-seven of which were available for analysis. Observations were made in one guinea pig in order to afford some basis of comparison of species and to exclude the possibility that our results were strictly or particularly peculiar to the cat; four fetuses were obtained, three of which were employed for analysis.

In preparing the cats, an anemic or ischemic decerebration was performed while the animals were under the influence of ether anesthesia. The basilar and the two internal carotid arteries were ligated and, the animals were then left undisturbed for 2 to 3 hours in order to allow time for the effects of the ether to completely wear off, thereby eliminating any narcotizing effects which might have been transmitted to the fetuses and which would have interfered with the initiation of respiration. A simple mid-line abdominal incision was made and the uterus brought to the outside. The guinea pig was prepared by exteriorizing the uterus under local cocaine anesthesia.

An incision of such magnitude and in such a position as would allow removal of one fetus was made in the uterus with as little trauma and hemorrhage as possible. At the moment the fetus was removed from the uterus, in most but not in all cases, 0.1 to 0.2 cc. of blood was gently withdrawn from the umbilical vein with a small hypodermic syringe and mixed with a small amount of heparin. Hemoglobin determinations were made with a Sahli instrument calibrated by the Van Slyke and Neil method. Breathing was prevented in one or two fetuses of each litter and the remainder of the litter were allowed to breathe for variable periods ranging from 1 to 24 hours. The animals which were to breathe no air were removed with the membranes intact and, while in this condition, their tracheas were clamped with small hemostats. After different lengths of time, the tracheas of those animals which had been allowed to breathe were clamped during inspiration.

The experiment on cat no. 4 (see table 1) was a single exception to the Caesarian section technique. In this case the fetuses were aborted at 58 to 60 days. Nevertheless, we were able to recover the fetuses with the membranes intact as they traversed the birth canal and to prevent respiration in two of them quite as effectively as in other experiments.

After clamping the trachea, the lung roots were exposed as quickly as possible by dissecting away the soft tissues and opening the bony cage with a pair of fine scissors. Care was taken to avoid cutting into the larger vessels, especially the internal mammaries, but enough of the thoracic cage was removed to facilitate tying the roots with little manipulation of the lungs. A strong silk ligature was placed around the root of each lung at a point where the pulmonary vessels and bronchi enter the lung substance, the roots were sectioned proximally and the lungs lifted out. Following very gentle washing with physiologic saline solution to remove any extraneous blood on the surface, the lungs were placed in small glass containers to await chemical estimation of iron content at our convenience.

We employed a method for estimating the total iron content of the blood-filled, ashed lungs which was, with some minor modifications, that worked out by Kennedy ('27) and Elvehjem ('30) for iron in biological material. The principle of analysis is based upon the formation of an intensely red salt, ferric thiocyanate, the absorption of this by amyl alcohol and the colorimetric comparison of this unknown material with a known standard containing 0.2 mg. of iron. It was thus possible to obtain quantitative values for the total amount of iron in the fetal lungs.

Since hemoglobin contains 0.335% iron the total iron in milligrams could be expressed in terms of hemoglobin by simple conversion. One very obvious error was introduced in attempting to express all the iron of the blood-filled fetal lungs in terms of hemoglobin. The tissues of the lung must contain some iron but the amount in fetuses is unknown. Bogniard and Whipple ('32) found it to be only about 6 to 7

TABLE 1
Summary of the estimations of iron and of blood in cat lungs before and after respiration at birth

CAT NO.	INSEMINATION AGE	FETUS NO.	BREATHED	ASHED LUNG WEIGHTS	TOTAL IRON	MG. FE/GM. ASHED LUNG	BLOOD Hb	TOTAL GRAMS Hb†	GRAMS Hb PER GRAM ASHED LUNG†	TOTAL in lungs	BLOOD VOLUME Per gram ashed lung
	days			gm.	mg.		gm. per 100 cc.			cc.†	cc.†
1	50	4	No air		0.20		10.3	0.0597		0.58	
2	60	2	1 hour		0.17		9.9	0.0507		0.51	
2		1	No air		0.101		10.3	0.0302		0.293	
2		2	1 hour		0.107		11.1	0.0319		0.287	
3	58	1	No air	0.050	0.231	4.62	9.85*	0.0689	1.38	0.699*	14.01*
2		2	1 hour	0.050	0.205	4.10	9.85*	0.0612	1.224	0.621*	12.43*
3		3	4 hours	0.045	0.208	4.63	9.7	0.0621	1.38	0.64	14.22
6		6	No air	0.050	0.242	4.84	9.85*	0.0722	1.44	0.733*	14.62*
							(3 & 4)				
4	58-60	1	2 hours	0.127	0.45	3.54	6.0	0.131	1.05	2.1	17.5
2		2	No air	0.074	0.308	4.16	8.1	0.092	1.24	1.13	15.3
3		3	No air	0.096	0.424	4.41	6.97*	0.126	1.31	1.8*	18.7*
							(1, 2 & 4)				
5	62-64	1	No air	0.121	0.416	3.43	11.65*	0.123	1.02	1.05*	8.75*
2		2	1 hour	0.103	0.400	3.88	11.9	0.119	1.15	1.00	9.66
3		3	1½ hours	0.071	0.345	4.85	11.4	0.103	1.44	0.903	12.63
4		4	2 hours	0.066	0.363	5.50	11.65*	0.109	1.64	0.935*	14.07*
							(2 & 3)				
6	62-64	1	No air	0.056	0.287	5.12	12.7	0.085	1.53	0.67	12.04
2		2	24 hours	0.063	0.333	5.28	11.7	0.099	1.56	0.846	13.33
3		3	1 hour	0.66	0.357	5.41	11.1	0.107	1.61	0.964	14.5
7	65	1	1 hour	0.066	0.38	5.75	13.7	0.113	1.716	0.825	12.52
		2	No air	0.069	0.49	7.10	12.7	0.146	2.119	1.150	16.68
3		3	1¼ hours	0.088	0.37	4.20	12.7	0.110	1.253	0.866	9.86
4		4	No air	0.084	0.46	5.47	13.2	0.137	1.633	1.038	12.37
8	63	1	1 hour	0.039	0.339	8.69	13.4	0.101	2.59	0.754	19.33
2		2	No air	0.045	0.412	9.15	12.7	0.123	2.73	0.968	21.49
3		3	1 hour	0.042	0.449	10.69	13.4	0.134	3.19	1.00	23.81
9	67	2	1 hour	0.028	0.315	11.25	12.0	0.094	3.36	0.783	28.00
3		3	No air	0.034	0.294	8.65	12.0	0.088	2.58	0.733	21.50

† Assuming all the iron to be in hemoglobin; tissue iron is unknown but probably is not more than 20% of the total.

* Averages of hemoglobin for other members of the litter; see text.

mg. for 100 gm. wet lung weight in healthy adult dogs. Even there it varied considerably. If tissue iron is relatively as great in the healthy fetuses of cats our estimations of total volume of blood in the lungs are all too high by perhaps 20%. Since we were interested primarily in comparative figures and not necessarily in actual volumes this error is of relatively little consequence to the present experiments.

RESULTS

All the chemical estimations of total iron and the computed amounts of hemoglobin and of volumes of blood in the experiments on cat fetuses are summarized in table 1. They form the basis for comparison of the animals which breathed and those which did not.

TABLE 2
Results obtained in one guinea pig, insemination age 63 days

FETUS NO.	BREATHED	FRESH LUNG WEIGHTS	TOTAL IRON	MG. Fe PER GM. FRESH LUNG	TOTAL Hb†	GM. Hb PER GM. FRESH LUNG†
		<i>gm.</i>	<i>mg.</i>		<i>gm.</i>	
1	1 hour	1.07	0.176	0.164	0.0525	0.0489
2	No air	1.61	0.184	0.120	0.0549	0.0358
4	$\frac{1}{2}$ hour	1.36	0.193	0.142	0.0576	0.0424

† See footnote, table 1.

Hemoglobin determinations were not obtained in all the fetuses. When not available, an average reading was computed from the values determined for the remainder of the litter. Such computed averages are designated by an asterisk in table 1 and the numbers of the fetuses; hemoglobin values for which were included in the computation, are placed in parentheses beneath these averages. The values for the total volume of blood and for the volume of blood per gram of ashed lung were computed from these average hemoglobin values and are therefore similarly designated by an asterisk. There were no hemoglobin determinations made on the guinea pig fetuses (see table 2).

In order to present a clearer and more direct comparison of the values obtained, we have rearranged the data into total averages for the animals that breathed air and for those that did not. In table 3 all the available figures were employed in computing the averages (excluding those for the guinea pig). The figures representing averages obtained by inclusion of the aforementioned estimated hemoglobin values are again designated by an asterisk. Table 4 represents the averages

TABLE 3

Comparison of average values in apneic cat fetuses with average values in air-breathing kittens at birth. All experiments are included

	TOTAL IRON [†]	MG. Fe PER GM. ASHED LUNG	TOTAL Hb [†]	GM. Hb PER GM. ASHED LUNG [†]	BLOOD VOLUME	
					Total in lungs [†]	Per gm. ashed lung [†]
	mg.		gm.		cc.	cc.
No air	0.322	5.695	0.0959	1.695	0.9036*	15.546*
Breathed	0.3195	5.983	0.0951	1.782	0.8690*	15.527*

* See footnote, table 1.

* See footnote, table 1.

TABLE 4

Comparison of average values limited to experiments in cats 6, 7, 8 and 9

	TOTAL IRON	MG. Fe PER GM. ASHED LUNG	TOTAL Hb [†]	GM. Hb PER GM. ASHED LUNG [†]	BLOOD VOLUME	
					Total in lungs [†]	Per gm. ashed lung [†]
	mg.		gm.		cc.	cc.
No air	0.389	7.098	0.1159	2.118	0.9118	16.816
Breathed	0.363	7.324	0.1083	2.183	0.8626	17.336

* See footnote, table 1.

of results obtained in the last four cat experiments only. These experiments were complete in every way and did not entail the use of any estimated values for hemoglobin readings. Moreover, the technique had become more nearly perfect in this latter group and it was thought that the results might be of greater significance than those in table 3.

DISCUSSION

The decerebration method for preparation of the cats, chosen primarily to facilitate another study, was unquestionably the

best for our present purpose also, because it eliminated the inhibitory effects of an anesthetic. The results of the experiment on cat no. 5 illustrate how important this is. The decerebration in this case was incomplete and in order to control movements of the mother sufficient nembutal was given to bring about surgical anesthesia. The fetuses were heavily narcotized at delivery and we experienced great difficulty in initiating respiration in them. This is at variance with the results of others (Boucek and Renton, '31) who reported that rat fetuses were not completely anesthetized by sodium amytal administered to the mother. In the remainder of the experiments no anesthetics were given and the fetuses began to breathe very readily.

In order to afford some indication of the accuracy of our chemical method and therefore of the validity of our results, we thought it expedient to undertake several test runs. Five series of three samples each containing known amounts of iron varying from 0.1 to 0.3 mg. were treated in exactly the same manner, step by step, as were the ashed lung samples. The final amyl alcohol extracts obtained were compared colorimetrically with our standard solutions. Table 5 is a record of the results of these experiments. In table 6 are presented the averages obtained for the number of milligrams recovered, the average deviation from the known values, and the per cent error.

From a consideration of these results it is evident that our determinations of total iron are reasonably accurate especially in view of the fact that the quantities of iron contained in the unknown lung samples proved to be approximately 0.3 mg.; the error in estimations of such quantities was around 1%.

It seems certain that if any significant error exists in our results, it is not one of chemical analysis but is in the operative procedure used. It is next to impossible to obtain information of the present nature without disturbing physiologic conditions as they normally exist. Although we attempted to reduce this to a minimum, there was a certain small amount of unavoidable hemorrhage incident to our intrathoracic ap-

proach. It is uncertain whether or not this blood loss affected the volume of blood in the pulmonary circuit materially. The factor is at least constant both in those animals that breathed and in those that did not.

TABLE 5
Test of method

TRIAL	SAMPLE	KNOWN MG. OF IRON	MG. Fe RECOVERED
No. 1	1	0.1	0.124
	2	0.2	0.211
	3	0.3	0.303
	Standard	0.2	
No. 2	1	0.1	0.107
	2	0.2	0.214
	3	0.3	0.300
	Standard	0.2	
No. 1	1	0.1	0.105
	2	0.1	0.107
	3	0.1	0.103
	Standard	0.1	
No. 4	1	0.2	0.194
	2	0.2	0.191
	3	0.2	0.195
	Standard	0.1	
No. 5	1	0.3	0.294
	2	0.3	0.294
	3	0.3	0.301
	Standard	0.2	

TABLE 6
Average of tests

	AVERAGE MG. Fe RECOVERED	AVERAGE DEVIATION IN MG.	AVERAGE PER CENT ERROR
0.1 mg. samples	0.1092	0.0092	9.2
0.2 mg. samples	0.201	0.0090	4.5
0.3 mg. samples	0.298	0.0032	1.07

Possibly a more valid objection to the technique employed is the unavoidable interference with intrathoracic pressures necessarily occasioned by the opening of the thoracic cage. Nor is this factor the same in both groups. Inasmuch as no

negative intrathoracic pressure is built up in fetuses prior to respiration, it is unlikely that entering the thorax produced change in pressures in these instances. However, in the newborn litter mates which had breathed air and which had thereby begun to establish a negative intrathoracic pressure, opening of the thorax must have materially increased the pressure therein. By clamping the trachea in inspiration, we prevented collapse of the lungs but we were of course helpless to prevent an increase in intrathoracic pressure which may have resulted in some alteration in pulmonary flow either by acting directly on lung capillaries and alveoli or indirectly on the heart and great vessels.

Although realizing the limitations necessarily inherent in experiments of this nature, we believe there is some significance in the data we have obtained. Viewed individually there are experiments such as those in cat no. 5 in which there appeared to be a significant increase in total iron per unit weight of lung correlated with the advent of respiration. This was not found consistently for in others such as cat no. 4 the converse was true. Even after breathing 24 hours one fetus of cat no. 6 showed practically the same amount of iron in its lungs as there was in an apneic litter mate. Examination of tables 3 and 4 reveals the very interesting fact that the average figures for the two groups, those which breathed and those which did not, are nearly identical; there was no appreciable difference in number of milligrams of iron in the lungs. It follows that there was likewise no great increase in the quantity of blood in the lungs of those animals which were allowed to breath if we may assume that the amount of tissue iron, an unknown factor, is constant throughout.

Concerning the minute volume of the pulmonary circulation we have no reliable data in the cat. The heart pumps blood about as fast as it can without marked vagal inhibition at the end of gestation. It becomes somewhat slower in man during the birth process but speeds up again immediately afterward.

The rate of the beat then declines rapidly and is no greater than the fetal rate at the end of an hour. A more gradual slowing continues from this time onward (Wiggins, '23). Therefore it would seem that there is no permanently accentuated minute volume to the lungs coincident with the establishment of breathing.

If we accept the concept that there is no increase in the volume of blood flowing through the pulmonary circuit during the first hour or so after birth, we are forced to accept the view that there must of necessity be developed in the fetus a sufficient pulmonary circulation to adequately care for oxygenation pending the assumption of respiration. Most significant from this standpoint is Patten's ('33) observation

that the pulmonary arteries are about the same size as the umbilical arteries and the total cross-sectional area of the pulmonary veins about the same as that of the umbilical vein. The placental circuit adequately takes care not only of oxygenation, but also of food intake and waste elimination. If, therefore, the pulmonary vessels are carrying a circulation in any way consonant with their size, there is enough blood already going to the lungs before birth to care for oxygenation as soon as the lungs are ventilated.

Moreover, studies by Thoma in 1894 (Keibel and Mall, '12) and later by Mall ('06) and Flint ('06) have shown that arteries and veins are formed from capillary nets and represent a functional adaptation of the net to the demands of the circulation. In other words it is developmentally unsound to assume the existence of well-developed pulmonary vessels which contain a volume of blood well below their capacity. The size of these vessels depends entirely on the volume of blood coursing through them and is a resultant adaptation thereto. It was interesting in this connection to observe the condition of the pulmonary vessels and lungs in the fetuses used. The vessels were well developed and filled with blood both in those fetuses which had breathed and those which had not. The lungs of the fetuses which had breathed no air

gave the appearance of rather solid vascular organs resembling the liver, while the lungs in those which had breathed were lighter in color and more frothy in appearance. Such a gross examination would certainly lead one to suspect that there is an appreciable circulation through these fetal lungs.

Our present results find some confirmation in a small amount of data on the pig and human reported by Krafka ('33). He observed that there was a significant proportion of the total fetal blood in the atelectic lungs, finding it to be about 3% in the pig and more than 5% in one human stillborn fetus.

Although it is true that the pulmonary circulation is relatively as well as absolutely larger in the adult than in the fetus, we believe that this growth must be a gradual one and not attained by any sudden or dramatic changes at birth. Patten ('33) pointed out that the gradual increase in inflation of the lungs, the gradual reduction of the hemoglobin content of the blood and the gradual increase in bore of the isthmus portion of the aorta with a concomitant reduction of that of the ductus arteriosus are all indicative of and parallel the increase in volume and efficiency of the pulmonary circulation.

It is our opinion that the above conception is not necessarily inconsistent with the findings of Hamilton, Woodbury and Woods ('37) concerning fetal ventricular blood pressures. These workers have reported a reduction of both left and right ventricular pressures with the first inspiration, the right being lowered three or four times as much as the left; after respiration starts, systole and diastole continue to produce rises and falls but at a lower level. The blood pressure values which they give beneath their illustrations are not all in agreement with their statements in the text and would seem to indicate an increase in intraventricular pressures after respiration has begun. Barcroft ('38) found systemic pressure raised. Be that as it may, any relationship between pressure changes and immediate alteration in the volume of the pulmonary circulation was unproved experimentally by Hamilton and his colleagues. Our own observations and those of Krafka ('31) provide the only estimations of such volume.

SUMMARY

Estimations of the total iron content of the blood filled lungs were made by a chemical colorimetric method in twelve fetal cats and one fetal guinea pig which had not breathed and in fifteen kitten and two guinea pig litter mates which had been allowed to breathe for 1 to 24 hours. Calculations based upon these values and on estimations of blood hemoglobin revealed no significant increase in the volume of blood in the lungs correlated with respiration at birth. Baring the possibility of errors introduced in interferring experimentally with normal physiologic pressure changes in the thorax of the air-breathing specimens, it would seem that there is no sudden increase in the volume of the pulmonary circulation at birth as has previously been assumed. It appears that a circulation is already present in the lungs during late prenatal life which is wholly capable of caring for oxygenation pending the assumption of respiration.

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THE EFFECTS OF AN EXPERIMENTALLY INDUCED ENDOCRINE IMBALANCE IN FEMALE MICE¹

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ONE TEXT FIGURE AND THREE PLATES (NINETEEN FIGURES)

Absence of the luteal phase in the estrous cycle has long been thought to be the cause of endometrial hyperplasia, or Swiss cheese endometrium (Fluhmann, '31). This has been fairly well substantiated by the fact that endometrial hyperplasia can be produced by the injection of estrogens. However, since it is necessary to use large amounts of estrogens in order to produce these as well as other abnormal growths in the female reproductive tract, some question still remains whether these effects can be brought about by endogenous estrogen. It has seemed to the author that in order to clarify this point it would be necessary to induce experimentally an endocrine imbalance which would duplicate the effects of estrogen injections. By grafting testes at birth it is possible to produce an endocrine imbalance in rats (Pfeiffer, '36) in which no corpora lutea are formed and there is a continuous but slow release of estrogen from the ovaries, but no endometrial hyperplasia occurs. Therefore, in the present experiments inbred strains of mice were used since it was thought that the homogeneity of graft and host tissues might increase the effectiveness of the grafted testes and thus cause a further alteration in the equilibrium between the pituitary and the

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ovaries than was possible in the rat, which would bring about a continuous release of estrogen at a high enough level to produce endometrial hyperplasia. Interpretation of the results obtained was facilitated by the fact that the effects of estrogen injections have been well standardized on these strains of mice.

MATERIALS AND METHODS

Thirty newborn female mice from seven different inbred strains² received both testes from litter mate males in the neck region by the technique described for rats (Pfeiffer, '36). Vaginal smears were made daily from the time the vaginas opened until autopsy. The animals were not sacrificed until decided changes in the cycles indicated a definite hormone imbalance.

At autopsy, measurements were made of the uterus in its normal position before and after contraction. The reproductive organs and mammary glands were preserved in Bouin's fluid, the adrenals and pituitaries in Helly's solution, and the bones in 10% formalin. The vaginas, uteri, ovaries and oviducts were sectioned at 10 μ and stained with Ehrlich's hematoxylin and trieosin. The mammary glands were stained in toto with hemalum and counterstained with trieosin after sectioning at 10 μ . The adrenals and hypophyses were sectioned at 8 μ . The adrenals were stained with Ehrlich's hematoxylin and trieosin, and the hypophyses with a modification of Mallory's technique. One femur of each animal was preserved in formalin, decalcified in formic acid, sectioned at 8 μ and stained with Dominici's combination of eosin-orange-G-toluidin-blue. The other femur was ground to a thin section and bleached with hydrogen peroxide. The pelvic regions were boiled in water for 5 minutes, and the flesh was carefully dissected away. The pelves were then bleached in hydrogen peroxide for 24 hours and were dried with slight tension on the ligament to prevent excessive shrinkage.

² The inbred strains of mice used are listed in table 1. They were obtained from the colony of Dr. Leonell C. Strong, Department of Anatomy, Yale University School of Medicine.

GENERAL OBSERVATIONS

At puberty all of the animals showed some enlargement of the clitoris which indicated that the testis grafts were vitalized and were secreting male hormone. Within 20 days after the opening of the vagina a hormone imbalance had become evident in some of the animals. This was characterized in ten cases by a predominantly estrous condition of the genital tract and in five others by protracted periods of dioestrus. The vagina was absent in one female of the F strain which had received three testis grafts at birth instead of the usual two. After 1 month laparotomies were performed on two animals in each group, a biopsy was made of one horn of the uterus, and one ovary was removed for histological study. The uteri of the predominantly estrous group were quite similar to those of normal estrous animals with the exception that in the former there was a slight indication of endometrial hypertrophy. The uteri of the animals whose vaginal smears indicated a predominantly dioestrous condition were identical with the normal dioestrous stage. The ovaries under both conditions were somewhat smaller than in the normal animal but contained young as well as maturing follicles. In the predominantly estrous group it was evident that the follicles were capable of maturing and ovulating since corpora lutea were present, and in one case ova were found in the oviducts. However, the corpora lutea were small and atypical. They resembled the corpora lutea which are found in the same strains of mice after a few weeks of estrogen treatment. In the predominantly dioestrous group the ovaries were smaller than in the estrous group and follicular development was not as great. However, a few fairly typical corpora lutea were present which must have persisted from former ovulations.

Since it was thought that eventually the ovaries would no longer be capable of producing corpora lutea, further laparotomies were not performed until some of the animals had been in continuous estrus for more than a month. Biopsies at that time showed that the uteri of those animals which had remained most constantly in the estrous condition were

characteristically hypertrophied. Therefore, all of the experimental animals were autopsied at various ages between 250 and 408 days (table 1), even though six animals still had fairly normal smears and showed correspondingly normal genital tracts. Twenty-four animals showed constant estrus or predominantly constant estrous cycles. Their uteri varied from a condition of slight hyperplasia, only noticeable upon histological sectioning, to an immensely hyperplastic and metaplastic condition in which the cervix and horns reached a diameter four times that of the normal control animal (fig. 1). In only two cases was there any indication of pyometra.

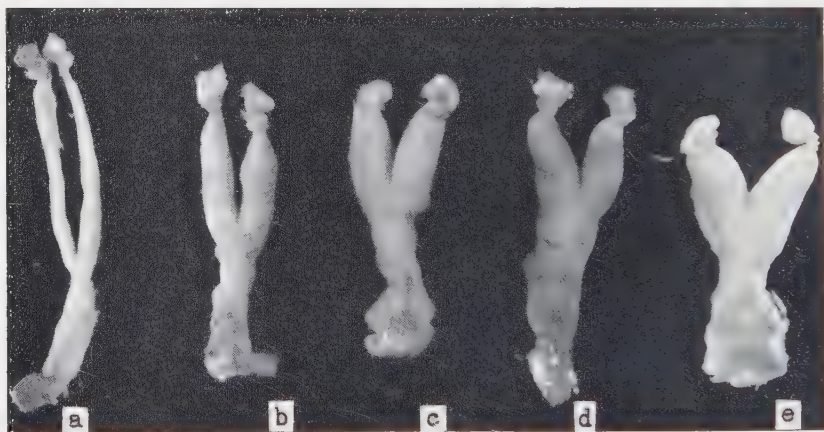


Fig. 1 Uteri photographed from alcohol after fixation in Bouin's fluid. (a) Normal dioestrous uterus; (b, c, d and e) progress of uterine hypertrophy as the endocrine imbalance continues. $\times 1.5$.

When a hyperplastic uterus was first exposed, it was observed to be of normal length but was hyperaemic and distended. However, immediately upon stimulation by mechanical or thermal (cold) means it contracted into a very compact rigid, V-shaped structure (fig. 1) whose diameter was almost as great as its length. The contractility increased in proportion to the amount of hyperplasia. In many cases there were small lumps or blisters under the serosa of the contracted uterus which caused it to have a mulberry appearance. The

TABLE 1

Effects of endocrine imbalance produced in female mice by testis grafts at birth

STRAIN	AGE AT AUTOPSY	ESTROUS CYCLE ¹	CONDITION OF THE OVARY ²	CONDITION OF THE UTERUS	TESTIS GRAFTS ³	PUBIC LIGAMENT	OSSIFI- CATION ⁴
	<i>Days</i>					<i>mm.</i>	
C ₃ H	375	PCE	Luteal-like cells	Hyperplasia	+++	2.5	+
C ₃ H	375	FNE	Normal	Slight hyperplasia	—	None	—
C ₃ H	375	PCE	Slight senility	Hyperplasia	—	>0.5	—
C ₃ H	375	PCE	Slight senility	Hyperplasia	++	>0.5	+
C ₃ H	375	IE	Enlarged rete	Slight hyperplasia	—	None	—
C ₅₇	286	IE	Rete cysts	Hyperplasia	+++	>0.5	+
C ₅₇	408	PCE	Luteal-like cells	Hyperplasia	—	>0.5	—
C ₅₇	281	PCE	Luteal-like cells	Extreme hyperplasia	+	>0.5	—
C ₅₇	402	PCE	Peripheral cords	Slight hyperplasia	+	0.5	+
C ₅₇	303	IE	Luteal-like cells	Fairly normal	+	0.5	+
C ₅₇	402	IE	Luteal-like cells	Hyperplasia	+	0.5	+
C ₅₇	402	PCE	Luteal-like cells	Slight hyperplasia	—	None	—
C ₅₇	401	PCE	Luteal-like cells	Hyperplasia	+++	2.0	++
C ₅₇	281	IE	Luteal-like cells	Slight hyperplasia	—	None	—
C ₅₇	281	CE	Luteal-like cells	Metaplasia, pyometra	+++	>0.5	+
I	275	CE	Luteal-like cells	Extreme hyperplasia	++	2.5	++
I	296	CE	Peripheral cords	Extreme hyperplasia	+++	3.0	+++
I	401	CE	Peripheral cords	Extreme hyperplasia	+++	5.0	+++
CHI	300	CE	Enlarged rete	Metaplasia	—	1.5	+
CHI	396	PCE	Peripheral cords	Orderly hyperplasia	—	1.0	+
CHI	345	CE	Enlarged rete	Pyometra	+	1.0	+
F	387	PCE	Enlarged rete	Slight hyperplasia	+++	None	—
F	250	*	Enlarged rete	Slight hyperplasia	+++	None	—
CBA	60	FNE	Normal	Normal	—	None	—
CBA	331	PCE	Luteal-like cells	Slight hyperplasia	+	>0.5	+
CBA	386	PCE	Luteal-like cells	Slight hyperplasia	+	>0.5	+
CBA	331	PCE	Luteal-like cells	Slight hyperplasia	+	>0.5	+
C	367	PCE	Normal corpora lutea	Cystic hyperplasia	+	1.0	+
C	367	PCE	Normal corpora lutea	Extreme hyperplasia	+	1.0	+
C	367	PCE	Normal corpora lutea	Hyperplasia	+	0.5	+

¹CE, constant estrus. PCE, predominantly constant estrus; showed varying numbers of leucocytes at times but always some cornified cells. IE, irregular estrous cycles having prolonged estrual stages with normal or prolonged periods of dioestrus between them. FNE, fairly normal estrus. * No vagina.

²The ovaries always contained some developing follicles but unless listed as normal they had a senile appearance, the corpora lutea were atypical, there were varying numbers of luteal-like cells present, and the rete portion was enlarged. Only the most striking feature of each ovary is given in the table.

³—, no grafted tissues recovered; +, only epididymis, vasa efferentia or sterile seminiferous tubules present; ++, seminiferous tubules containing spermatogonia; +++, well-organized seminiferous tubules containing primary or secondary spermatocytes.

⁴—, no bony spicules in the femur; +, macroscopically visible spicules; ++, well-developed spicules and a visible increase in the thickness of the compact bone; +++, marrow cavity of the femur completely obliterated by newly formed bone.

ovaries were always slightly smaller than those found in normal females, and always contained a few macroscopically visible follicles. However, only in a few cases was it possible to identify corpora lutea in the fresh material.

The pelvic symphyses were movable in most of the animals which showed hyperplasia of the uterus. The length of the pubic ligament was roughly correlated with the amount of hyperplasia, the degree of altered endocrine function, and the genetic strain of the mice (table 1). The most consistently well-developed pubic ligaments were found in the I strain where, in one case, the ligament reached a length of 5 mm. The next longest ligaments were found in the C₃H and C₅₇ mice, but they never exceeded 2.5 mm. Hypercalcification of the remainder of the skeleton occurred in all animals having pubic ligaments and could be directly correlated with the length of the ligament. In those animals having movable pelvises but ligaments too short to be measured only slight endosteal hypercalcification occurred, while in those having pubic ligaments 3 to 5 mm. in length the marrow cavity of the femur was almost completely obliterated by newly formed bone. The femurs were the only bones which were extensively studied in the present investigation since the osseous changes which occur in them following estrogen injections have been fairly well standardized. In extreme cases the extent of hypercalcification and pubic ligament formation was comparable to that obtained after 10 weeks treatment with 250 i.u. estradiol benzoate weekly. The remainder of the skeleton was affected to a slightly less degree.

Stimulation of the mammary glands occurred in all animals which showed an altered endocrine condition. This stimulation did not exceed that which may be produced by estrogen injections. In most strains it consisted only of duct development and the formation of a few alveoli. However, in the I strain secreting glands were obtained (figs. 19 and 20), but they did not produce milk (see p. 476). There was no indication of the development of mammary tumors in tumor resistant strains, nor did the incidence of tumors in susceptible strains

exceed that of multiparous females of corresponding age and strain.

HISTOLOGICAL OBSERVATIONS

1. *Ovaries.* The ovaries in the altered endocrine group were always smaller than those of the controls (fig. 3). They varied greatly in histological appearance, depending on the duration of the experiment and on strain differences. In all animals which did not show alteration in the estrous cycles the histology was normal. However, shortly after the onset of prolonged periods of estrus, in all except the C strain, the corpora lutea often appeared atrophic and became even less typical as the condition progressed, although the follicles and interstitial elements remained normal. In the C strain luteinization was normal or excessive even though the condition of the uterus indicated that there were marked endocrine disturbances. The fact that degenerating ova were seldom found in the larger follicles in any of the strains, while ova were sometimes present in the oviducts, indicates that ovulation occurred during the early stages of the endocrine imbalance.

When irregular cycles or constant estrus had been established for more than a month, practically all of the corpora lutea resembled the pale, apparently functionless, corpora lutea which persist after the beginning of estrogen injections. However, thecal luteinization was still evident (fig. 4). Pale luteal-like cells also appeared in the medulla of the ovary. These were probably interstitial cells which had become enlarged and had taken up lipoids. In some cases they were dispersed rather evenly throughout the medulla, but usually they were clumped into cyst-like formations which were often large and sufficiently well organized to resemble superficially corpora lutea. A similar luteinization occurred in the cells near the periphery of the ovary. These cells were of epithelial origin and were often arranged in indistinct cords (fig. 6). Sometimes the luteal-like cells contained a large amount of yellow pigment. This condition was often extreme enough to give a distinct yellow cast to the ovary. In the I

strain the large cysts of luteal-like cells often came to make up most of the ovary. The number of the luteal-like cells present could be correlated with the duration and degree of endocrine imbalance if strain differences were taken into account. The germinal epithelium was not very active (fig. 6) and the growth and maturation of follicles was slowed down proportionately to the increase of the luteal-like cells (figs. 3 and 4). However, it was never completely suppressed, and the follicles which matured did not seem to be atypical in any way and eventually ovulated. One of the animals showing a pronounced imbalance had four histologically normal ova in one of the oviducts. However, the corpora lutea which were forming in this animal were of the pale type and did not have a normal appearance. Probably the most consistent effect of continued endocrine imbalance noted was an increase in the size of the rete ovarii (fig. 5).

In the animals whose endocrine imbalance had continued for longer periods of time the ovaries resembled those of senile animals except for the presence of masses of luteal-like cells and the large size of the rete ovarii (fig. 5). Granulosa and true theca interna cells were greatly decreased in number in spite of the physiological evidence that a good supply of estrogen was being released. There was also a marked decrease in the number of oocytes present in the ovaries. In the C₅₇ strain there was a definite tendency toward the formation of fluid-filled ovarian cysts which are thought to have been of rete ovarian origin. In extreme cases they sometimes occupied two-thirds of the cross sectional area of the ovary at their centers.

2. *Oviducts.* During the first month of the endocrine imbalance the entire epithelium of the oviduct remained normal. After that time, the epithelium of the middle portion increased in height and eventually the nuclei became arranged in two or more layers, giving an appearance of stratification (fig. 7). However, this was probably a pseudostratification due to the extreme height of the cells, rather than a metaplasia. This condition has also been observed after extensive treat-

ment with estrogens. The cuboidal epithelium of the fimbriated end of the oviduct remained unaffected by the endocrine imbalance.

The reactions of the oviducts were apparently influenced by strain differences. In the C₅₇ mice the epithelium did not become as high as in other strains but frequently penetrated the muscle layers and formed blebs under the serosa, while the cytoplasm of the cells became rather extensively vacuolated. The oviducts of CHI mice tended to have extremely thick walls due primarily to unusually heavy muscular layers. In this strain the epithelium was often convoluted to such an extent that the lumen appeared lobulated. This appearance was undoubtedly due to contraction of the muscular layers. The oviducts were normal in all the animals which did not show alterations in the vaginal cycle.

3. *Uterus*. In all animals whose vaginal smears showed a condition of constant estrus or prolonged periods of estrus, hyperplasia of the endometrium occurred. This could be determined by laparotomy since the hyperplastic uteri exhibited great contractility in response to tactile or thermal stimuli. As the endocrine imbalance progressed, the uterus developed a cystic hyperplasia of the endometrial glands and metaplasia of the epithelium or, as occurred in two cases, pyometra. Pyometra never became very acute and consisted primarily of a heavy infiltration of leucocytes and a few macrophages. When there was no alteration in the vaginal cycle, the histology of the uterus was typical of the stage of the cycle at which biopsy was performed or at which the animal was autopsied.

The cystic hyperplasia varied considerably with both the duration and the degree of the endocrine imbalance (figs. 8, 9, 10 and 11). At first, endometrial glands developed only in the upper third of the uterus. Later the endometrium thickened and endometrial glands began to develop over the entire uterus. This development continued to such an extent that it was usually impossible to distinguish the lumen of the uterus from the many cystic ducts of the glands (fig. 10). During this

stage the glands came to occupy the entire endometrium and in many of the uteri pushed aside the bundles of muscle fibers and migrated into the connective tissue of the tunica serosa (figs. 10 and 13). In some cases they extended out under the serosa and formed blisters of varying sizes on the outer surface of the uterus (figs. 10 and 14), giving a mulberry appearance to the contracted uterus at the time of removal and fixation. The height of the epithelium in the glands depended on the amount of distention by secretion. However, in some of the uteri with extensive glandular hyperplasia the glands had a regular arrangement and did not extend into or through the myometrium (fig. 15). It was these uteri which usually showed the greatest metaplastic tendency (fig. 11). Near the cervixes glandular overgrowths occurred which in two cases were possibly beginning adenomata (fig. 12). Except for this overgrowth, the endometrium was quite uniform throughout its length. In one case transitional epithelium consisting of stratified cylindrical cells extended throughout the entire uterine lumen, and no glands were present (fig. 9). This epithelium was similar to that found in the upper portion of the cervix. The stroma was appreciably hypertrophied, and in some cases hyalinized areas appeared under the epithelium of the ducts of the glands. These areas first underwent liquefaction and then became filled with amorphous, lightly staining material. This hyalinization was more pronounced than in normal animals of the same age but did not occur around the blood vessels, nor did it develop sufficiently to be treated as foreign material by the connective tissue.

4. *Cervix*. In the animals having an endocrine imbalance the cervix hypertrophied (fig. 16), but it did not show as great an increase in size as did the uterus because of the absence of cervical glands in the mouse. However, in extreme cases of uterine hypertrophy the endometrial glands migrated down to the junction with the vagina, giving the false impression that cervical glands were present. Their openings, however, always remained above the cervix. There was usually some hyperplasia of the cervical muscle layer and an increase

in all the connective tissue components. Hyalinized areas similar to those occurring in the uterus were also present in the stroma of the cervix near the epithelium.

In all the animals which were in a state of constant estrus the vaginal end of the cervical epithelium was cornified. The extent of this cornification varied with the degree of endocrine imbalance. The cornified epithelium merged into a heavy layer of transitional epithelium which extended upward until it in turn gave way to the typical columnar epithelium at the point where active growth or budding of the endometrial glands began. The transitional epithelium was never observed to give rise to endometrial glands even though it sometimes extended into the uterus. Epithelial whorls occurred frequently in the C₅₇ strain. Many of these whorls were rather large and sometimes pushed into the underlying connective tissue.

5. *Vagina*. In all of the animals which showed an endocrine imbalance the vaginal epithelium was heavily cornified (fig. 17). There was a general hypertrophy of the vagina and an increase in the amount of connective tissue. However, there was no indication of excessive hyalinization. The vagina resembled in all ways the condition found when constant estrus is produced by injection of estrogens. In the C₅₇ strain epithelial whorls similar to those occurring in the cervix in this strain were also present in the connective tissue of the vagina (fig. 17).

6. *Testis grafts*. At autopsy, tissue (presumably the testis grafts) was removed from the site of grafting in twenty-one cases. Ten of these proved to be good testis grafts. They contained interstitial elements and seminiferous tubules with Sertoli cells, spermatogonia, and usually primary and secondary spermatocytes. Some of these grafts reached an even higher stage of development than a 7-month cryptorchid testis. This was probably due to the lower temperature of a graft under the loose skin of the neck. The remaining eleven grafts contained only vas deferens or epididymis or a few sterile tubules. The vas deferens and epididymis persisted much

better than the testis itself. Where testis tubules and interstitial elements remained the epithelium of the vasa efferentia was of the tall, functional type, indicating that the graft had been secreting male hormone (fig. 2). In some grafts the epididymal tubules near the testis were stimulated by the male hormone produced by the graft, while those some distance away were heavily cornified due to estrogen present in the circulation of the host. In other grafts the epithelium appeared active throughout the epididymis. Thus there was apparently a great variation in the amount of male hormone that was being produced by the grafts.

7. *Mammary glands.* The mammary glands of the I strain approximated very closely those of lactating animals. They showed complete duct development and alveolar growth (fig. 20). Secretion had begun and the ducts were distended with fluid. However, this fluid was not true milk. In the other strains of mice the ducts of the mammary glands were relatively long but they showed little tendency to arborize nor did much alveolar growth occur.

8. *Hypophyses.* In the animals which showed the greatest uterine disturbance the hypophyses were slightly larger than in the normal animal. This was most consistently true of the C₅₇ strain in which the pituitary bodies increased about one-third in size. The hypertrophy shown in the C₃H strain was slightly less. There was no trace of tumor formation. Although cell counts were not made on the hypertrophied hypophyses, a great increase in the number of chromophobe cells was readily evident. However, proliferation of chromophobe cells was too slow to be detected by any marked increase in mitosis.

9. *Adrenals.* The adrenals did not seem to be very much affected by the endocrine imbalance, even in those cases where the most extreme stimulation of the uterus occurred. However, a well-developed X-zone persisted (fig. 18) and there was possibly a slight general hypertrophy.

10. *Thyroids and parathyroids.* No condition was found in the experimental group which did not occur as frequently in control mice.

11. *Bone.* After the endocrine imbalance had continued for some time the pubic symphysis began to loosen. At this time ground sections of the femurs showed the beginning of hyperossification. Both processes progressed until in extreme cases the bones appeared solid, and a pubic ligament several millimeters in length was formed. These two conditions were closely related to each other and were influenced by strain differences and the length of time the endocrine disturbance was in effect (table 1). However, the hyperossification appeared to lag somewhat behind the formation of the pubic ligament. In extreme cases the bones appeared solid, but in histological sections it was seen that, particularly in the center, considerable marrow remained imprisoned between the heavy spicules. The hyperossification appeared to start with spicule formation at the epiphyseal end and to migrate toward the center of the shaft. The forward growth of the spicules progressed relatively more slowly than did the osteoblastic activity near the old endosteal border. Thus, the spicules had very heavy bases. Where the spicules interlaced, the marrow between them became trapped to form small islands surrounded by compact bone. These islands were gradually filled in with bone, and others were formed more centrally by the same process. This process caused a gradual thickening of the compact bone of the shaft. The new bone had the same organization as the original compact portion of the femur. The epiphysis remained open under all circumstances.

DISCUSSION

The alteration in the endocrine system described here differs from that produced in the rat by the same operative technique (Pfeiffer, '36) in that corpora lutea sometimes form. This indicates that preovulatory growth occurs and, therefore, the estrogen level may be somewhat higher than in the rat, which may possibly account for the great hypertrophy of the reproductive tract which occurs. However, the mechanism which brings about the altered endocrine condition is probably the same in the mouse as in the rat, since in

both cases an equilibrium was established between the ovary and the hypophysis which resulted in a continuous rather than a cyclic production of estrogen, as evidenced by constant stimulation of the reproductive tract. The altered equilibrium was undoubtedly due to a masculinization of the pituitary body similar to that observed in rats and was dependent upon the early establishment of the testis graft (Pfeiffer, '36). The fact that luteinization may occur in the mouse does not detract from this hypothesis since it also occurs in ovaries grafted into male mice. However, under both circumstances the corpora lutea formed are pale and appear inactive, which suggests that they secrete little, if any, progesterin. This would explain their non-interference with the continuous production of estrogen. Since there is no correlation between the occurrence of endocrine imbalance in the female and the condition of the graft at autopsy, it is evident that there is no antagonism between the testis graft and the ovary other than the competition of each to impress its type of equilibrium on the hypophysis. However, masculinization of the pituitary body is not the only means by which a state of equilibrium may be produced between the hypophysis and the ovary such that a continuous release of estrogen occurs. Direct intrinsic damage to the ovaries of the guinea pig by external means such as treatment with a minimum sterilization dose of x-rays (Schmidt, '39) or subtotal castration (Lip-schütz, '37 a and b, '38) also causes a continuous production of estrogen and effects on the reproductive tract similar to those described in the present paper.

Although it is necessary to inject large amounts of estrogen in order to bring about changes in the genital tract such as those described above, it is evident that the level of endogenous estrogen produced by the ovaries of mice whose pituitary bodies had been masculinized was probably not very high, even though there was a great hypertrophy of the reproductive tract, since there was less than the normal amount of theca interna present. The fact that smaller amounts of estrogen were required when the hormone was produced

endogenously is not surprising when one considers that, above a certain minimum quantity necessary to bring about a reaction, the effectiveness of hormones is dependent on the constancy with which they reach the receptor organs. Therefore, minute amounts of endogenous hormone released continuously over long periods of time may be just as effective as extremely high doses administered by weekly, or even daily, injections. It has been suggested by Schmidt ('36) that estrogen may be secreted by certain masses of luteal-like cells which she has observed in the ovaries of guinea pigs following treatment with a minimum sterilization dose of x-rays. Similar luteal-like bodies were present in the ovaries of mice which had received testis grafts at birth. However, in both experiments theca interna cells, generally recognized as a source of estrogen (Corner, '38), and granulosa cells were present. Therefore, it remains possible that these cells were producing all of the estrogen secreted. The luteal-like masses have been described as forming from the thecal cells around the degenerating follicles in guinea pig ovaries following x-radiation (Schmidt, '39) and subtotal castration (Lip-schütz, '37 a and b, '38) and in rat ovaries which were transplanted to the ears of males (Deansley, '38). Thecal luteinization was also seen often in the ovaries described in the present investigation and probably contributed in part to the formation of the luteal-like bodies. However, clumps of luteal-like cells, ranging in size from two cells up to the complete corpus luteum-like body, were so numerous in the medullae of some of the ovaries that it seems improbable that all of the aggregations could have been formed from thecal cells. The cells in the smallest groups resembled very closely deficiency cells which have been activated by APL (Selye, Collip and Thomas, '33) in the ovaries of hypophysectomized rats and were probably derived from the so-called interstitial cells. The luteal-like cells which occurred at the periphery of the ovary and were often arranged in indistinct cords were probably similar to those which Brambell and Parkes ('27) found in adult mice following x-ray treatment. The fact that

there was no hyperactivity of the germinal epithelium suggests that they were derived from anovular cysts in the manner described by Brambell and Parkes and had not migrated in as secondary cords from the epithelium.

The changes which occurred in the genital tracts of mice with masculinized pituitary bodies resembled very closely those which follow the continued injection of large amounts of estrogen (Gardner, Allen and Strong, '36; Loeb, Suntzeff and Burns, '38), with the exception that no true cancerous growths occurred. However, it is possible that cancerous growths might have appeared had these animals been allowed to live until natural death. In order to test this point it will be necessary not only to allow mice having an endocrine imbalance to live until natural death but also to use large numbers of animals, since the incidence of these growths in estrogen treated animals is very low. It is evident, however, that at least most of the pathological changes which follow extreme estrogen treatment are not the result of a pharmacological action caused by the presence of estrogen in amounts greatly exceeding those which could possibly be produced by the ovary, but rather are conditions which can just as readily be caused by endogenous hormone provided an endocrine imbalance between the ovary and the hypophysis has been produced such that the estrogen is released continuously.

As might be expected, there were certain minor differences between the conditions resulting from an endocrine imbalance and those following estrogen injections which may be correlated with the age at which stimulation began and the length of time over which it acted, or may be explained by strain or individual differences in the mice used. In general the hypertrophy and hyperplasia shown by the genital tract were the same in both cases. However, in mice having an endocrine imbalance there was not as much hyalinization of the stroma as in mice which had received estrogen injections (Gardner et al., '36; Loeb et al., '39). On the other hand, the hyperplasia of the endometrial glands was more uniform. The glands penetrated into and through the myometrium more fre-

quently and had a greater tendency to migrate out under the serosa. In one case stratified epithelium extended the full length of the uterus, indicating that the endocrine imbalance had either begun at a very early age (see Gardner et al., '36) or that the metaplasia was a reaction to inflammation (Gardner and Allen, '37). It is interesting that endometrial glands were absent in this uterus. This suggests that glands will not arise from a stratified epithelium. Stratified epithelium has, however, been found in the ducts of endometrial glands (Loeb et al., '39), but it was probably the result of a metaplasia which occurred after the glands had developed. The hypertrophy shown by the hypophyses of animals having an endocrine imbalance was similar to that which follows prolonged administration of estrogen but was never of the adenomatous type described by Cramer and Horning ('36) and Burrows ('36) which occurs rather rarely (Gardner, '37). The extent of hypertrophy varied in the different strains but was greatest in those strains which are most affected by estrogen injections. The influence which strain differences may have was most strikingly illustrated by the responses elicited in the mammary glands where, depending on the strain, development ranged from simple duct formation to the production of secreting alveoli. The same responses in these strains have been obtained by estrogen injections (Gardner, '37, and unpublished).³ The rete cysts which occurred in the C₅₇ strain are probably also strain-limited. There was no evidence that these cysts have any endocrine function.

The effects which the endocrine imbalance produced on the pubic symphysis were the same as those which follow estrogen injection (Gardner, '36). Since the length of the pubic ligament was roughly correlated with the degree of endometrial hyperplasia, it seems probable that, as in mice receiving estrogen injections, the extent of the pubic ligament is indicative of the estrogen level. The hypercalcification which oc-

³ The author was given permission by Dr. William U. Gardner to examine his slides of the mammary glands of the various strains of mice after estrogen treatment. Glands of all except the C strain were available.

curred in the long bones was also identical with that which follows estrogen treatment in the mouse (Gardner and Pfeiffer, '38). However, it differed markedly from the hypercalcification produced in the bird (Pfeiffer and Gardner, '38; Kirschbaum, Pfeiffer, Van Heuverswyn and Gardner, '39) in that well-organized compact bone was formed instead of the spongy type characteristic of the bird. This difference may be due either to the rapidity with which the bone is formed in the bird or to some other species difference.

SUMMARY AND CONCLUSIONS

1. An endocrine imbalance between the hypophysis and the ovary was established in female mice by grafting testes from litter mates at birth. This imbalance caused a constant production of estrogen by the ovary which in turn induced great hypertrophy of the genital tract.

2. The production of the endocrine imbalance was dependent on the early vitalization of the testis graft. However, after the imbalance had become established, the condition of the graft, which at autopsy varied from only a few sections of vasa efferentia to well-developed seminiferous tubules, was apparently unimportant.

3. The ovaries had a premature senile appearance and usually contained large numbers of luteal-like cells which were often organized to resemble corpora lutea. A few developing follicles were always present and ovulation sometimes occurred. However, the corpora lutea which were produced were pale and atypical.

4. The hypertrophy of the genital tract involved all elements but was particularly manifested by an increase in the height of the epithelium in the middle portion of the oviduct, a tremendous hyperplasia of the endometrial glands which often resulted in the glands penetrating the myometrium and even extending out under the serosa, the formation of hyalinized areas in the uterine horns and cervix, and a heavy cornification of the epithelium of the vagina and lower portions of the cervix. The epithelium of the cervix and of localized areas

of the uterine glands underwent metaplasia. Growths which were possibly adenomatous appeared near the cervix in two cases.

5. The mammary glands developed to the same stage as that obtained by estrogen injection.

6. The pelvic symphyses were resorbed and replaced by ligaments. These changes were accompanied by extensive hyperossification of the long bones.

7. The hypophyses were enlarged by about one-third. This was due to an increase in the number of chromophobe cells. The adrenals still retained the X-zone. The thyroids and parathyroids were normal.

8. It is concluded that any of the effects produced by the injection of large amounts of estrogen may be brought about by endogenous hormone provided an adequate endocrine imbalance has been established. The mechanism by which the endocrine imbalance was produced and by which extensive hypertrophy of the genital tract was maintained, even though the ovaries did not appear hyperactive, is discussed.

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PLATES

PLATE I

EXPLANATION OF FIGURES

All figures are photomicrographs of histological preparations stained with Ehrlich's hematoxylin and counterstained with tricresin.

2 Testis graft 375 days after transplantation into the neck of a litter mate female. Note the tall columnar epithelium in the vasa efferentia. $\times 170$.

3 Ovary of a C_{57} mouse which had been in constant estrus for 90 days. Note the small size of the ovary, the scarcity of large follicles, and the open medullary area. $\times 20$.

4 Higher magnification of a portion of an ovary from a C_3H mouse after prolonged periods of estrus showing thecal luteinization around a degenerating follicle and numerous luteal-like cells both dispersed through the stroma and organized into bodies which resemble corpora lutea. $\times 85$.

5 Portion of an ovary of a CHI mouse after prolonged periods of estrus. Note the open hylus and the hypertrophy of the rete ovarian region. $\times 85$.

6 Periphery of the ovary of a CHI mouse after prolonged periods of estrus. Note the inactive germinal epithelium and the pseudo-cord arrangement of luteal-like cells. $\times 170$.

7 Oviduct of an I mouse showing extensive hyperplasia and pseudostratification of the epithelium. $\times 85$.

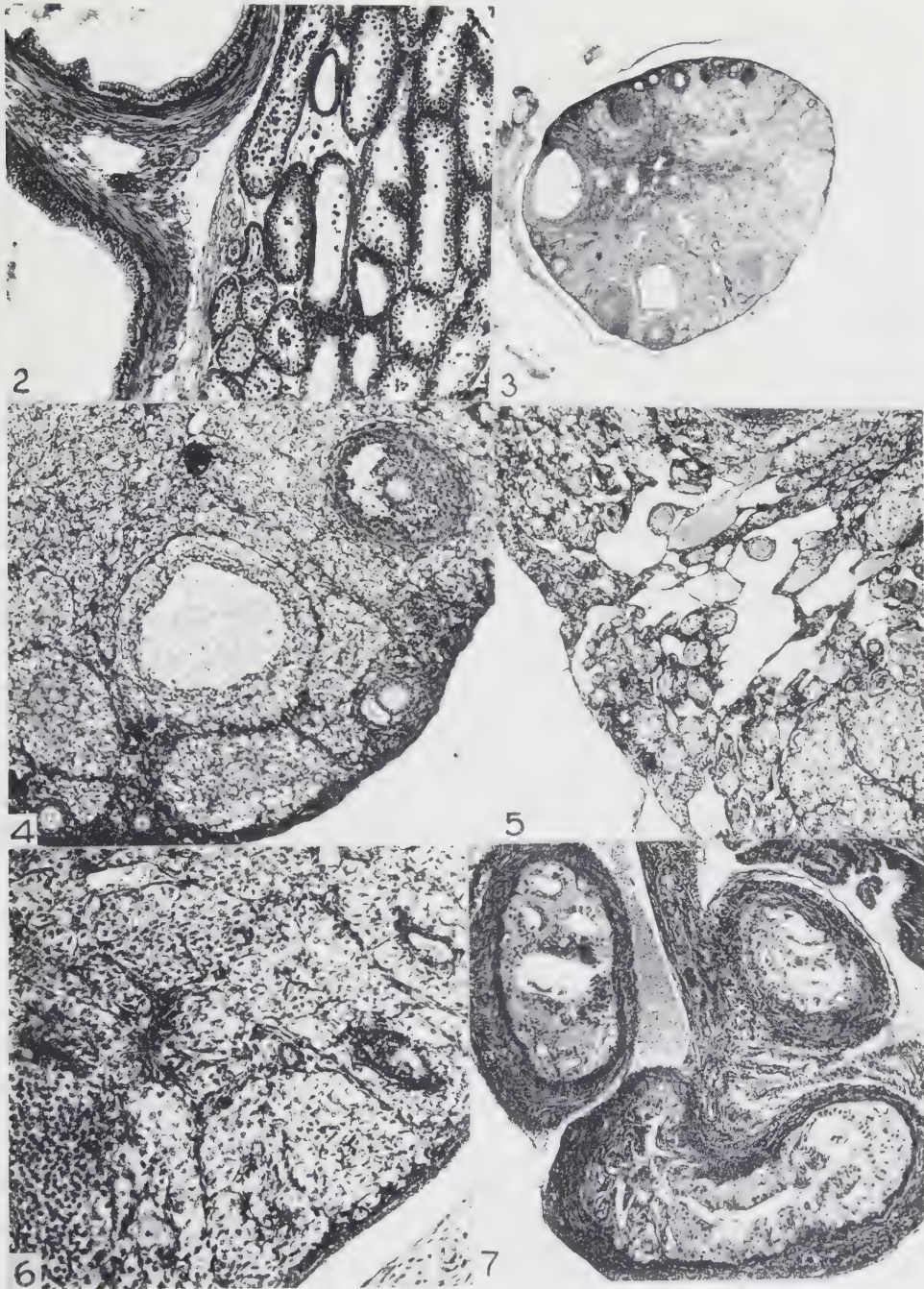


PLATE 2

EXPLANATION OF FIGURES

All figures are photomicrographs of histological preparations stained with Ehrlich's hematoxylin and counterstained with tricrossin.

8 Cross section of the uterus of a CBA mouse at the beginning of endocrine imbalance (prolonged dioestrous and oestrus stages). This is fairly typical of the normal suboestral condition. $\times 20$.

9 Cross section of the uterus of a CHI mouse which had very long periods of constant estrus. Note the metaplasia of the endometrial epithelium and the absence of endometrial glands. $\times 20$.

10 Cross section of the very hyperplastic uterus of an I mouse. The cystic ducts of the greatly hypertrophied endometrial glands cannot be distinguished from the lumen of the uterus. Note that some of these cystic glands extend under the serosa. The peculiar shape of the cross section is due to the contraction of the cystic uterus upon handling and fixation (see text). $\times 20$.

11 Cross section of the uterus of a C₃H mouse showing tremendous general hypertrophy and extensive metaplasia of the endometrial glands. However, the glands are not cystic. $\times 20$.

12 A growth (possibly adenomatous) which appeared in the endometrium near the cervix of a C₃H mouse which had extreme hyperplasia and metaplasia of the uterus. $\times 85$.

13 Endometrial gland which is growing through the myometrium. $\times 85$.

14 Endometrial gland which has extended under the serosa and has become cystic so that it forms a bleb on the surface of the contracted uterus. $\times 85$.

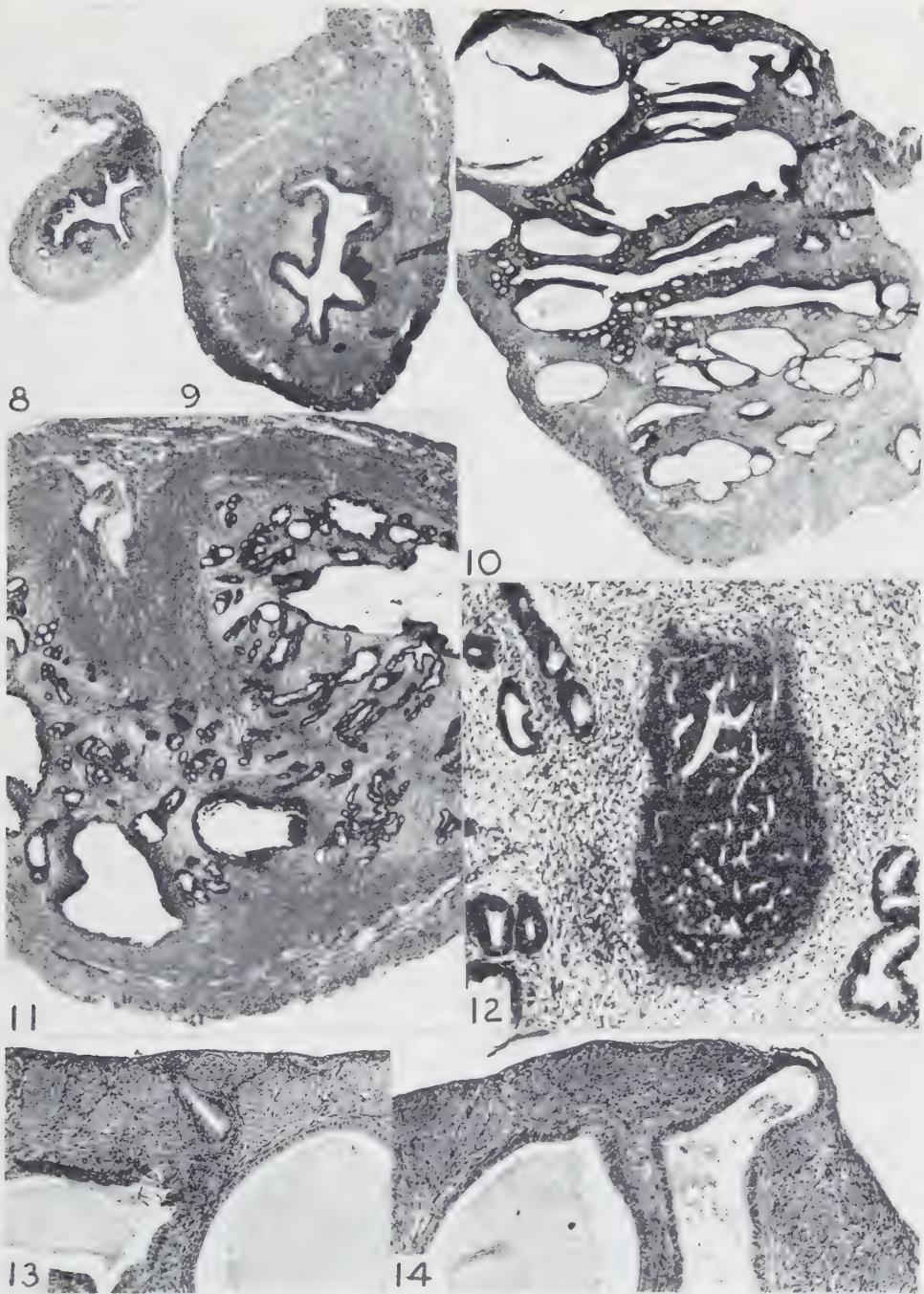


PLATE 3

EXPLANATION OF FIGURES

All figures except 19 and 20 are photomicrographs of histological preparations stained with Ehrlich's hematoxylin and counterstained with tricresin.

15 Portion of a cross section of a greatly hypertrophied uterus from an I mouse showing endometrial glands which have a regular arrangement. Note the beginning of hyalinization of the stroma. $\times 85$.

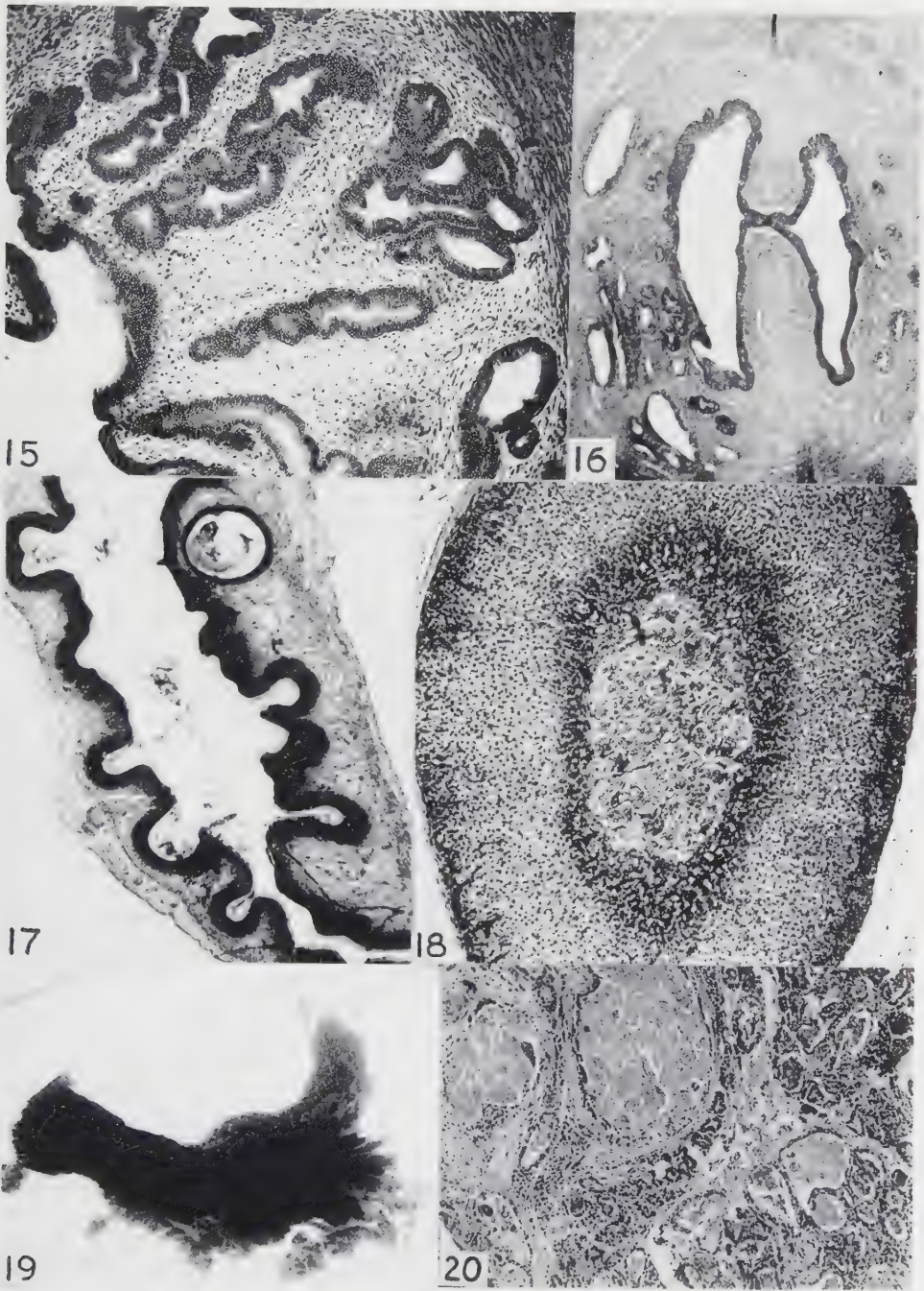
16 Cross section of the upper end of the cervix of a CBA mouse showing heavily stratified epithelium and general hypertrophy of the stroma. $\times 20$.

17 Cross section of the vagina of a C₅₇ mouse showing heavily cornified epithelium. Note the epithelial whorl in the submucosa. $\times 20$.

18 Cross section of the adrenal gland of a C₃H mouse at 365 days of age which had an endocrine imbalance for several months. Note that the X-zone is still well developed. $\times 85$.

19 Shadowgram of the right second mammary gland of an I mouse which was 401 days of age and had been in constant estrus for several months. Whole mount, hemalum. $\times 2.5$.

20 Cross section of the left secondary mammary gland from the same I female as in figure 19. Note the large amount of secretion in the cystic ducts. Photomicrograph, hemalum and tricresin. $\times 85$.



STUDIES ON DIRECT AND VISIBLE INGESTION OF FAT BY DIFFERENTIATED BODY CELLS OF THE CAT

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TWO PLATES (ELEVEN FIGURES)

INTRODUCTION

Particulate fat absorption by intestinal columnar epithelium was suggested by Munk (1884), but current teachings postulate hydrolysis in the lumen of the small intestine and resynthesis within the cells.

Recent studies on the behavior of cells in taking up and releasing fat, led us to believe that this physiochemical process is more physical than chemical, and does not require complex chemical reactions (Singer and Zwemer, '34; Grynfeldt, '37; Zwemer, Wotton and Norkus, '38).

Some of the technical difficulties in the histocytological investigation of fats have been overcome (Zwemer, '33), so that we can now offer further details on the entry, and to some extent the subsequent distribution of fats in the body after a meal.

PROCEDURE

Normal young adult cats were used. The animals usually had fasted for 24 to 36 hours before the experimental feeding, but some were allowed to eat salmon before receiving the fat. A measured amount (see table 1) of melted beef fat, melted crisco, cod liver oil, olive oil or mineral oil was administered by means of a stomach tube. The various fats had previously been saturated with Sudan IV for later identification. The animals always settled down and became contented and quiet after feeding.

After desired time intervals (table 1) each animal was sacrificed and immediately autopsied. Illuminating gas was employed as the lethal agent, to eliminate the use of fat-solvent anaesthetics. In one case ether was found to produce no material difference in the histological picture. In every case, with the exception of the animal given mineral oil, the wall of the small intestine was pink in color and the mesenteric lymphatics were seen as red channels. Red stained fat

TABLE 1
Animals and tissues studied

ANIMAL NUMBER	WEIGHT IN KILOGRAMS	ABSORPTION TIME	QUANTITY AND TYPE OF LIPOID	TIME SINCE LAST FEEDING
		<i>hours</i>		<i>hours</i>
3	2.3	1	10 cc. Cod liver oil	24
11	..	1	20 cc. Olive oil	24
10	3.3	1½	30 cc. Beef lard	2
4	2.1	1½	15 cc. Cod liver oil	24
9	3.8	1¾	30 cc. Beef lard	2
18	4.0	1¾	60 cc. Cod liver oil	36
1	..	2	5 cc. Cod liver oil	..
12	..	2	30 cc. Olive oil	24
2	2.0	2	10 cc. Cod liver oil	24
15	3.8	2	50 cc. Vegetable fat ¹	12
8	2.3	2½	30 cc. Beef lard	2
7	2.9	2½	30 cc. Beef lard	24
14	..	2½	30 cc. Olive oil	24
6	2.1	2¾	30 cc. Beef lard	24
5	3.3	3	30 cc. Beef lard	24
13	..	3	30 cc. Mineral oil	24
16	2.6	3	50 cc. Vegetable fat	12
17	3.0	4	50 cc. Vegetable fat	12

¹ Crisco.

streamed from the thoracic duct when it was cut. Tissues for microscopic study were fixed in 10% pyridine formalin pH 7.0 (Burke, '36). Ligation was employed before removal of segments of the intestine, pieces of the lungs, liver, lymph nodes, spleen and receptaculum chyli to prevent loss of fat from lumina and blood vessels.

Blocks of fixed tissue were carefully washed in running water over night to remove the fixative, infiltrated with gelatin

(Zwemer, '33), and frozen sections from 10 to 40 μ in thickness were cut with a sliding microtome. Sections spread by floating on distilled water were then transferred and fixed to slides. Consecutive sections of each block were stained as follows: the first section was mounted directly with no stain other than that already in the fat; the second was dipped in Sudan IV, to intensify the stain in the fat given and to stain additional fat possibly present; the third was double stained with hematoxylin and Sudan IV. Various cytoplasmic counter stains such as Pontamine yellow and INT blue in aqueous solution were also tried and yielded good results. The hematoxylin and Sudan combination was the most satisfactory as well as the most pleasant to the eye. All sections were mounted in Glychrogel (Zwemer, '33; Wotton and Zwemer, '35).

OBSERVATIONS

In the lumen of the small intestine the fats were seen as large masses and globules adhering closely to the tips of the villi. Any hydrolysis or emulsification that may take place probably serves only to break down connective tissue and free the inclosed fats. Droplets ranging in size from those small enough to lie within the striations of the cuticular border to ones many times the volume of a columnar cell, were observed in and next to these cells all along the sides of the villi. Large drops in process of absorption have an 'hour-glass' appearance. One bulb lying in the gut lumen is connected to the other within the cell by a hair-like constriction passing between the striations of the cuticular border (fig. 2). Some of the very small droplets of fat seem able to pass through the cuticular border without constriction.

The columnar cells become loaded with fat drops, some of which may be as large as their nuclei. The fat passes on through the basement membrane in the characteristic 'hour-glass' formation, the globules enter the blood capillaries apparently by passing between the overlapping edges of the endothelial cells (figs. 4 and 5). Some columnar cells discharge their fatty contents into interstitial spaces forming

the core of the villus, and finally drain in the central lacteal (fig. 6).

Those droplets, which upon leaving the columnar cells enter the capillary loop, are quickly carried away by the swiftly moving blood stream. This probably accounts for the transportation of a large proportion of the absorbed fat and the rapidity of its removal may explain why it has seldom been observed in these capillaries. The remainder of the fat in the central lacteal (figs. 4 and 6) is squeezed by the contraction of the villus (Starling, '33) into the submucosal lymphatic plexus.

Occasional granular cells were seen in the villi but their small number would not account for much of the fat transferred. Most of them were eosinophiles which even during active fat absorption showed no tendency to pick up or carry lipid material (fig. 5). The possibility of fat transportation by histiocytes and other cells as proposed by Schafer ('12) is slight.

The experimental fat meal ranged from 10 cc. to 50 cc. for each animal. Maximum visible absorption was reached in from $1\frac{1}{2}$ to $2\frac{1}{4}$ hours after feeding. Absorption begins as soon as the fat reaches the small intestine, regardless of the presence of other food substances, and continues until all of the lipid has disappeared from the intestinal lumen. Smaller quantities and lower melting-point fats are in general taken in more rapidly. With higher melting-points and larger quantities, absorption proceeds at a slower rate but the histological picture is similar. Beef fat appears more finely divided than cod liver oil. The lower viscosity of the oil probably enables cells to absorb larger droplets. This might explain the so-called 'absorption by drops' and 'absorption by streams' phenomena of Mottram, Cramer and Drew ('22).

Frozen sections of lung show fat in the cells of the capillary walls. Zinnser ('23) has reported active phagocytosis of foreign matter by these endothelial cells. The first capillary bed reached by the fat entering the lacteals and thence by way of the thoracic duct to the veins is that of the lung alveoli.

TABLE 2

Fat in gastrointestinal tract after feeding

NO.	TIME (HRS.)	AMOUNT OF FAT (CC.)	LUMEN	CUTICULAR BORDER	COLUMNAR CELLS	CENTRAL LACTEAL AND CAPILLARIES
3	1	10	In large masses and droplets of varying sizes	Heavily loaded with fine droplets on surface and within striations. Also large masses adherent to border	Heavily loaded, fat drops mainly in the cuticular end	Heavily loaded and congested with fat
10	1½	30	In large masses, large and moderate-sized drops. Other food	Large drops and masses interspaced with smaller drops adherent	Evenly and finely emulsified, mainly in cuticular end	Frequent moderate-sized drops
4	1½	15		Few small drops in the striations	Most of the fat is in the anterior end. Many drops can be seen passing through the basement membrane	Actively carrying fat
9	1½	30	Occasional masses composed of moderate-sized drops. Other food	Apparently free of fat drops in most places	Filled with moderate-sized drops evenly distributed	Congested with fat in droplet form
1	2	5		Border practically free of lipid. Similar to 1½ hours	Anterior ends of cells packed with large fat globules	Drops of varying sizes and finely divided particles
2	2	10		Same as no. 1 (2 hours)	Same as no. 1 (2 hours)	Same as no. 1
8	2½	30	Other food	Little droplets in the striations	Well filled with medium-sized drops	Apparently negative
7	2½	30	Empty	Occasional small-sized drops in striations	Large pieces resembling the Golgi apparatus and finely emulsified particles	Negative. Fat drops mixed with RBC in the collecting ducts in the region of the crypts of Lieberkuhn
6	2¾	30	Empty		Scattered remnants and fragments of drops and fat in a state of very fine emulsification	
5	3	30	Empty	Border free of fat	A few fragments of drops left, resembling the Golgi apparatus	Central lacteal collapsed. Most of the fat is in the collecting lacteals in the region of the lamina propria

We have not determined the speed of appearance or disappearance of the fat in the lung.

In the liver, fat was seen in the sinusoids in droplets form, many of the droplets as large as red blood cells. The particles of fat pass from the blood stream into the cytoplasm of the parenchymatous cells. That a definite force is exerted in the

TABLE 3
Fat in liver and receptaculum chyli

ANIMAL NUMBER	AMOUNT OF FAT	LIVER	RECEPTACULUM CHYLI
10	1½ hrs. 30 cc.	Cells loaded with fat in large areas around central veins. Most hepatic cells have the 'signet-ring' appearance. Active fat absorption	Negative
4	1½ hrs. 15 cc.		Lymph channels filled with scattered phagocytes containing cellular debris and fat particles
9	1¾ hrs. 15 cc.	Fat in areas around central veins composed of cells with fat in drops and fragments resembling the Golgi apparatus	Lymph channels loaded with large and small drops frequently coalescing
2	2 hrs. 10 cc.	Most cells in area show 'signet-ring' condition. Areas often confluent	
8	2¼ hrs. 30 cc.	Areas of fat absorption around central veins reduced. Cells filled with medium-sized drops	Fat in lymph channels in drops of moderate size well distributed
7	2½ hrs. 30 cc.	Large areas around central veins of cells filled with medium- and small-sized drops, resembling the Golgi apparatus	Heavily congested channels filled with solid confluent masses of fat
6	2¾ hrs. 30 cc.	Areas of hepatic cells heavily loaded with finely emulsified and occasional drops	Lymph channels heavily loaded with large separate drops of fat
5	3 hrs. 30 cc.	Similar to no. 6 but emulsification much finer	Lymph channels occasionally congested with small to fine-sized drops

ingestion of these fat globules can be seen by the stretching of such globules across sinusoids between two cells (figs. 7, 8, 9). Hepatic cells will take up fat until they resemble adipose cells (fig. 11), and the hepatic lobule fills with fat from the central vein toward its periphery (fig. 10). This ingestion differs very little from that observed in the columnar absorbing epithelium.

DISCUSSION

Our observations suggest that there is little difference in the absorption of high or low melting-point fats of plant and animal origin. The fats are absorbed by the columnar cells wherever the two come in contact, and so long as there is fat to be absorbed.

This fat, visibly unchanged, passes by way of the lumen to the small intestine through the columnar, absorbing cells into the body. Its subsequent transportation and distribution is shared by both the blood and lymphatic circulation. The fat droplets taken in and carried by the venous drainage from the mesenteries directly reach the hepatic portal system. Those picked up by the central lacteals of the villi pass through the thoracic duct into the left subclavian vein, and after first passing through the pulmonary capillaries and systemic circulation, enter the liver through the hepatic artery (chart 1).

Whether carried by the lymphatics or directly by the blood stream, a major portion of this fat ultimately reaches the circulation of the liver. Here it is withdrawn from the blood stream by the parenchymal cells and incorporated into their cytoplasm (pl. 2).

As Zinnser ('23) has pointed out in his discussion on phagocytes, "The most primitive form of digestion is an intracellular one." The morphology of the evolutionary development of the division of labor is expressed by the differentiated tissue systems and organs of the higher forms. In the single celled animals one unit of life performs all necessary functions, but in the metazoans specialized activities have been assumed by individual tissue-cell groups. In certain cases

of emergency (phagocytosis of materials foreign to the body) and for normal processes many cells of the mammalian body retain their fundamental function of direct ingestion. This phenomenon is not confined to any one group of free ameboid cells. Fixed tissue cells and all mesenchyme deriva-

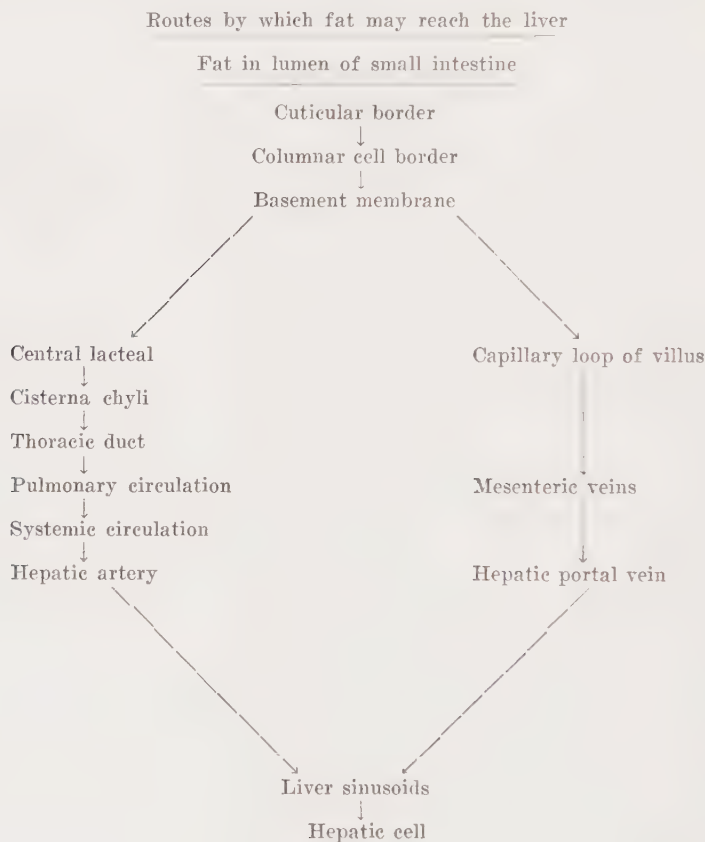


Chart 1

tives seem to possess the potentialities for phagocytosis (Aschoff, '24; Linton, '29; Gay, '35). In lower forms such as the coelenterates and planaria phagocytosis of solid food particles by the intestinal epithelium can be clearly demonstrated. Our evidence suggests that in the mammals the

endodermal derivatives (intestine, lung, liver) may retain their fundamental property of phagocytosis in connection with fat digestion.

This property of direct mobilization of visible fat drops by cells of the body is not confined to the digestive tract or phagocytes or ingestion. During secretion, cells of the female breast have been shown by Grynfeldt ('37) to push out large globules of fat. Singer and Zwemer ('34) have observed the apparent secretion of lipid droplets by living adrenal cells. The presence of fat in the blood stream, and a method for counting the droplets was reported by Gage ('24) 15 years ago.

We have not determined the chemical identity of the fat in the liver cells with the type administered by stomach tube; so that the experiments reported here do not exclude their having been changed by enzyme activity. Newer aspects of the chemistry of fat metabolism have been recently reviewed by Sperry ('39).

The penetration of oil drops into living cells has been studied by Chambers and Kopac ('36, I and II) and their illustrations show the phenomenon in free living cells. They found that the "size of the oil drop which penetrates under a given condition is inversely proportional to the tension at the oil/aqueous-phase interface." Surface active substances such as certain bile constituents would markedly lower the tension at the surface of oil drop in the intestine. A discussion of the physical chemistry involved, can be found in the papers by Chambers and Kopac, and that of Danielli and Harvey ('35).

SUMMARY

The ability of differentiated, adult mammalian cells to take in fat in visible particulate form has been investigated.

Cats fed a meal of fat, previously stained with Sudan IV, were sacrificed at selected intervals. Gelatin-embedded frozen sections were then made of intestine, liver, lung and other organs.

Our observations indicate that fat droplets pass through the cuticular border of intestinal columnar cells, traverse the cells and leave at the basement membrane into the marginal capillary. The drops in the blood capillaries are carried directly to the liver by the portal system. There the parenchymatous liver cells take them up, sometimes becoming so engorged as to resemble adipose cells.

Some of the fat in the villi does not enter the capillaries, but reaches the central lacteal. This fat goes to the thoracic duct by way of the receptaculum chyli, and passing through the chambers of the right heart reaches its first capillary bed in the lungs, where some fat is taken up.

The administration of fats of high or low melting points of plant or animal origin made no essential difference in the histological picture. Mineral oil did not pass through the columnar cells.

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PLATE 1

EXPLANATION OF FIGURES

1 Intestinal epithelium showing fat droplets in contact with the cuticular border and a suggestion of two strands going into a cell from the center drop. (Green filter.) $\times 2400$.

2 Same as figure 1 but a different focus, showing fat passing in a fine stream through the cuticular border; 'hourglass' drop. (Blue filter.) $\times 2400$.

3 Low power view of intestinal epithelium, showing same droplets as in figures 1 and 2. (Green filter.) $\times 250$.

4 Drops of fat entering central lacteal of villus apparently squeezing between endothelial cells of lacteal. (Green-yellow filter.) $\times 720$.

5 Intestinal epithelium showing large droplets of fat in the marginal capillary and some columnar cells, but none in the granular leucocytes. (Yellow filter.) $\times 1200$.

6 Villus showing fat passing through the columnar epithelium into the central lacteal. This figure from a section of the intestine of a toad given a fat meal and killed by pithing, is included because the cell size is larger than the mammal and the experimental conditions were similar to those described for the cat. (Yellow filter.) $\times 1200$.

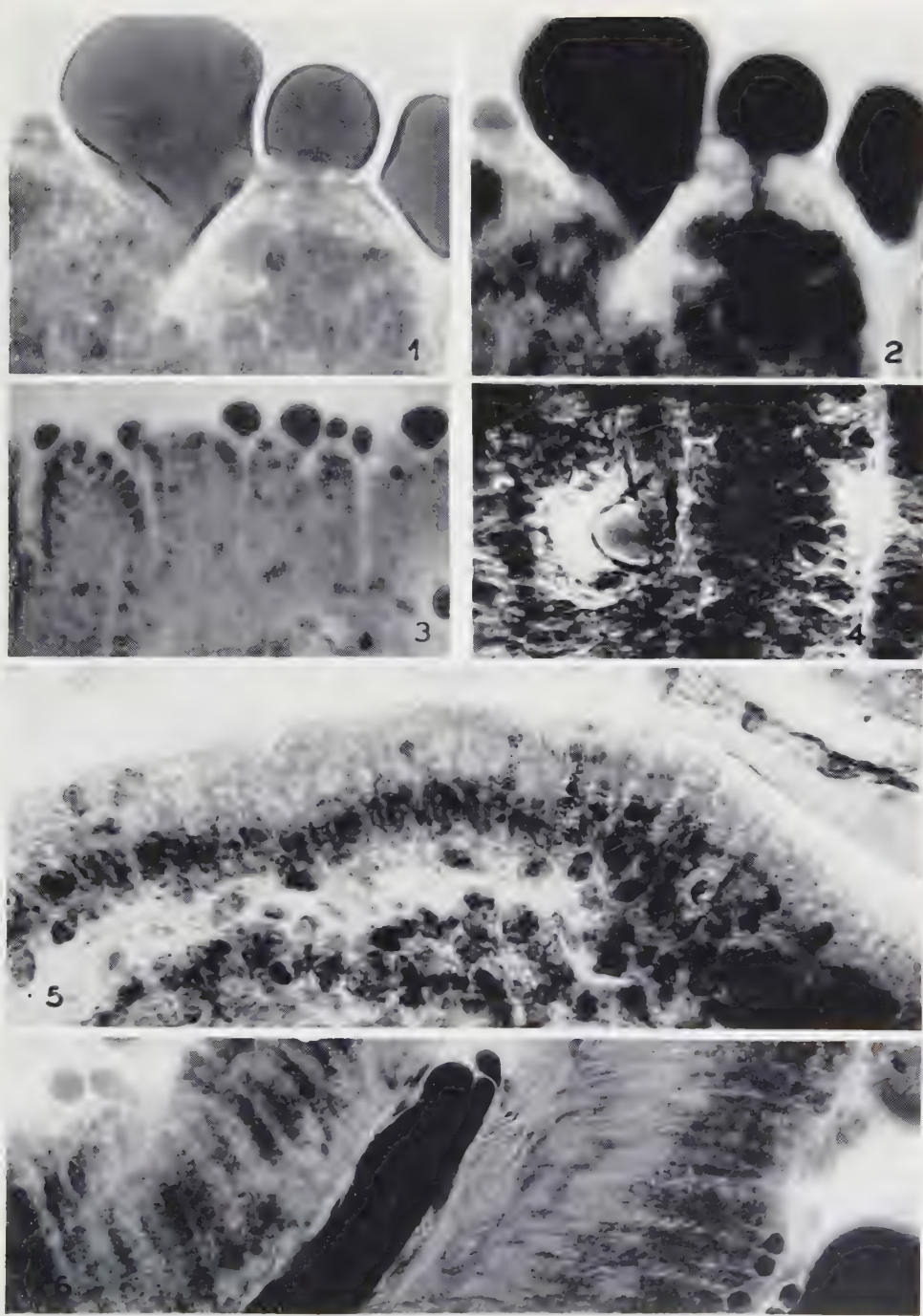


PLATE 2

EXPLANATION OF FIGURES

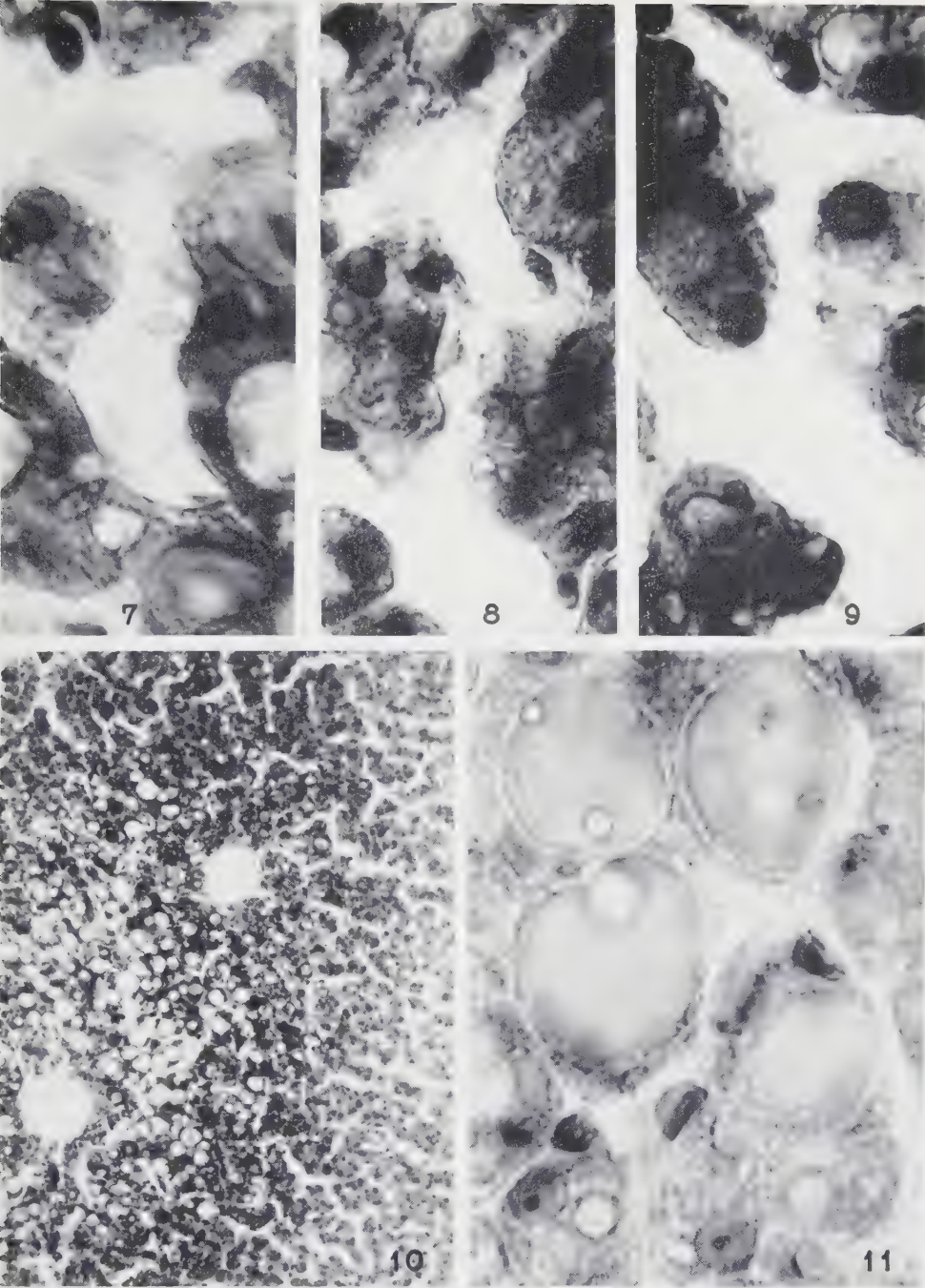
7 Hepatic sinusoid, fat drop being drawn between opposite cells. (Green-yellow filter.) $\times 1200$.

8 Hepatic sinusoid, showing two drops, one being drawn between two cells and the other entering a single cell. (Green-yellow filter.) $\times 1200$.

9 Hepatic sinusoid, in which opposite cells have almost divided and ingested a single fat globule leaving a narrow strand. (Green-yellow filter.) $\times 1200$.

10 Low power of hepatic lobule showing cells around central vein filled with fat. (Green-yellow filter.) $\times 120$.

11 Hepatic cells heavily loaded with fat giving a 'signet-ring' appearance similar to typical adipose cells. (Green-yellow filter.) $\times 1200$.



AN UNDESCRIBED TYPE OF PARTIAL SEX REVERSAL IN DOVE HYBRIDS FROM A SUB-FAMILY CROSS

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ONE TEXT FIGURE AND TWO PLATES (FIFTEEN FIGURES)

It is now well known that certain representatives of the two families, Columbidae (Common pigeon ♂) and Peristeridae (Ring dove ♀), may be crossed and that only or practically only male offspring result. It is also known that the numerous sperm produced by the resulting hybrids are of double normal size (in consequence of the omission of the second spermatocyte division in their formation) and otherwise abnormal (Guyer, '00; G. Smith, '09). To that group of facts the present study incidentally adds a demonstration of still other repression of development in the testes of such family hybrids—these testes grow more slowly and attain distinctly smaller size than do these organs in either parent species.

Crosses made between certain genera of Peristeridae have been shown to yield classes of offspring in which sexuality and reproduction capacity vary according to the particular genera employed (Whitman, '19; Ghigi, '29; Taibel, '30; Riddle, unpublished data). Some representatives of Zenaidura and Zenaida (sub-family Zenaidinae) may cross with undiminished fertility, and their offspring are fully fertile inter se and with (at least) pure Zenaidas, while the hybrids from some species of Turtur and Streptopelia (sub-family Turturinae) show limited fertility and certain other aberrant sex phenomena. Connected with these variations are moot questions concerning the validity of several genera—some of which

would be reduced to specific rank by some authorities (Hartert, '21; Selater, '24; Taibel, '34). In general, however, functional male and female offspring appear in all these 'generic' crosses and, apart from the fact that both hermaphroditism and entire absence of gonads frequently appear in later generations, the germ glands of these groups offer little that is useful for histological study.

Special opportunity for histological study of development of an unusual type of gonads is afforded regularly, however, by the 'not-male' hybrids which result from a cross (of subfamily rank) of the male Mourning dove (*Zenaidura macroura carolinensis*) with the female Ring dove (*Streptopelia decacota* or *risoria*). The present paper is mainly concerned with the nature of the aberrant gonads possessed by a minority of these hybrids—gonads which macroscopically simulate ovaries in the embryo and newly-hatched birds but which are rudimentary and macroscopically of doubtful nature in adults. At hatching this tissue is very largely restricted to the left side, and at this and all later stages of life there is a small unpaired left oviduct. These facts suggest but do not prove that these birds are genetic females; for a cytological determination of this and related points suitable material was collected by Dr. Robert T. Hance whose report is not yet available. There were fifty-one hybrids possessing this type of gonad in fraternities which yielded eighty-three males and sixty-nine additional embryos which were either killed for study or died in early stages of development. Infertile eggs were rare during that part of the year (May to September) when the Mourning dove is capable of producing sperm. The male hybrids possess essentially normal testes except that their growth is abnormally slow and their adult size is smaller than is characteristic for birds of like body weight.

Since the results of our histological study of the abnormal (female) gonads indicate repression of ovarian development at all stages—with tendency to form testicular tubules in more advanced stages—it is also thought useful to present data showing other aspects of 'repressed development' in both

males and 'females' of this fraternity and likewise in the solely male offspring of the 'family' hybrids first mentioned above. Data for these latter hybrids show that both their body weight and testis weight is unexpectedly small; in the sub-family hybrids, which are here our chief concern, the available data show that body weight, heart weight, and rate and ultimate amount of testis growth are all repressed. Such widespread repression of growth in other gonadal and somatic tissues should assist an understanding or interpretation of the strong repression which is associated with partial sex reversal in that minority of sub-family hybrids which start the development of ovaries.

MATERIAL AND METHODS

The gonads of twenty-one sub-family hybrids (δ *Zenaidura* $\times$ f *Streptopelia*), ranging in age from hatching (14 or 15 days after the egg is laid) to slightly over 3 years, have been subjected to thorough histological study. Altogether the gonads of more than 150 of these particular hybrids have been examined either macroscopically, by means of fixed material, or by both methods. Except in adult males the gonads were very small and it was therefore usually necessary to remove the entire gonadal region, including persistent mesonephros, adrenal and gonad for the present study; in these cases the weights of the gonads were not obtained. Zenker's and Bouin's fixing fluids have been employed and serial sections were cut from 6–8 μ in thickness followed by staining with hematein, Heidenhain's hematoxylin, and Mallory's triple stain.

HISTOLOGY OF THE ABERRANT GONADS

From hatching to age of 1 month. At hatching (15 days after egg is laid) and for some time thereafter only a left gonad having the shape of an ovary is present; in structure (fig. 2) this organ grossly resembles the ovary of a normal 8- to 9-day Ring dove embryo (fig. 3). Cortical and medullary regions are distinguishable and a thin tunica is present (fig. 2).

The cortex is literally a thickened germinal epithelium consisting of several layers of cells which vary from cubical to columnar in shape. The germinal epithelium is actively forming epithelial cords which extend into the medulla. These cords are apparently homologous with those of the second proliferation—the cortical cords of the normal ovary. The cells comprising them seem to be entirely epithelial in nature since no germ cells can be identified definitely; it is quite possible, however, that some germ cells are present but are not sufficiently normal in structure to permit their identification by the usual criteria. At neither this nor any later time in the development of these gonads do we find any cells undergoing presynaptic stages such as one finds in normal gonads. The germinal epithelium likewise reveals no germ cells—at least none which are normal in appearance—although comparable stages of normal ovaries show many germ cells in the cords of the second proliferation.

In gross appearance the medulla is likewise quite comparable with that of a normal ovary from an 8- to 9-day dove embryo, with exception that the loose appearance of the medullary cords, due to their expansion, is perhaps more accentuated than in the normal. Isolated cells which in size resemble germ cells are found scattered among the epithelial cells which comprise the medullary cords (fig. 4). A comparison with similar cells found in the normal ovary of the Ring dove shows this striking similarity (fig. 5). Perhaps these are germ cells which were brought down by the first proliferation forming the medullary cords, though possibly they are enlarged epithelial cells from the medullary cords. This latter possibility is supported by observations on older gonads in which some of these epithelial cells apparently hypertrophy and simulate germ cells in respect to size.

Within the medullary region are found also a few solid cords varying in size according to the number of cells comprising them. One of these is shown in figure 2. The smallest specimens of this type consist of a cluster of only two or three cells, but these are undoubtedly formed from cells of the

medullary cord. To some extent they resemble very young definitive testicular cords, and from the standpoint of their origin they would of course be homologous with them.

It is evident therefore that at the time of hatching both the gross appearance and the histology of these putative ovaries demonstrate their true ovarian nature, and also evident that already at this early stage of development these immature ovaries show retarded development and an obvious lack of normal germ cells.

We have representative stages of ovaries from birds killed at 15–30 days after hatching. During this time of rapid bodily growth the left gonad increases somewhat in size and lengthens considerably, extending along the entire ventral surface of the adrenal. The germinal epithelium remains thickened, is moderately active, and forms solid epithelial cords. Additional cords are also being formed by clustering and apparently by multiplication of medullary cord cells. In some cases, however, such ‘ovaries’ from birds 15 days after hatching show very few of these cords. At all early ages isolated cells characterized by a clear cytoplasm are found in the medulla; these may be somewhat disguised germ cells. It is by multiplication of these and of the surrounding small epithelial cells that some of the solid cords arise deep in the medulla.

At all early stages these left gonads remain extremely small and show great retardation in development although they retain a moderately active germinal epithelium. The undeveloped left oviduct is present throughout life.

Gonads from birds 4.8 months and 5.5 months of age. The ovary-like gonads of birds of these ages are slightly larger than those of 15-day birds but they show extreme retardation in development in comparison with normal gonads. The germinal epithelium remains thickened and is actively forming solid cords which extend a short distance into the medulla (figs. 6 and 7). In a normal Ring dove of corresponding age one finds a fairly well developed ovary with ova of 0.5 mm. in diameter or larger. The abnormal gonads of sub-family hybrids, however, still resemble their condition at hatching

(fig. 2) and are entirely devoid of developing ova, even though cords of the second proliferation have been forming since hatching. Such cords are entirely abortive, and the ovary-like gonads thus show very profound retardation of development insofar as ovarian tissue is concerned.

The epithelial cords which originate in the germinal epithelium become surrounded by a basement membrane and by one or more concentric layers of loose connective tissue. The cells of most of the cords usually have no definite arrangement but are scattered at random throughout—often having no contact with the basement membrane. They have clear or lightly granular cytoplasm and rather large nuclei, but no definite germ cells are found among them. A few cords develop lumina which in some instances become filled with cellular debris (fig. 7). In their incipient stages the cords can be seen within the germinal epithelium as clusters of cells (fig. 6). These clusters increase in size and migrate through the loosely formed tunica into the underlying medulla where they may grow considerably in length and diameter.

Besides the cords which arise from the thickened germinal epithelium other cords form also deep in the medulla as derivatives of the medullary cords (those of the first proliferation). In figure 2—a gonad at hatching—one such cord can be seen rather far down in the medulla, and older gonads show more of them. The presence in our material of a series of such cords—varying from a cluster of two or three cells to very large cords—leads to the conclusion that they arise by rearrangement and mitotic activity of the epithelial cells composing the medullary cords. Although the two kinds of cords (primary and secondary) have different origins they are histologically indistinguishable after being scattered through the medulla.

Gonads from birds 1 to 3 years of age. When 6 to 12 months of age—the time when normal birds would attain sexual maturity—the abnormal left gonads of most of these hybrids show very marked histological change and become more testis-like. In adult or nearly adult hybrids this gonad shows a

maximum development of primary and secondary cords; these cords then almost completely fill the gland, replacing the loosely appearing medullary region which was quite prevalent in younger stages (figs. 8, 9 and 10). At this time many cords have enlarged and a few have lumina; none, however, shows any gametogenic activity. One is impressed with the resemblance of these cords to young definitive testicular cords (compare figs. 11 and 12). All intergrades between smaller and larger cords are present (figs. 8, 9 and 11). It is thus evident that gonads which were essentially retarded ovaries at the time of hatching have become more testis-like during that later period which normal avian gonads utilize for a spurt of differentiation and growth. Macroscopically also these gonads have become somewhat more cylindrical in appearance and thus more testis-like in shape. In a few cases, however, this type of gonad was found to be still more completely retarded; even in a 12-month bird we have observed that in most respects this gonad may resemble its condition at hatching. The average weight of eleven of these abnormal left gonads from adult hybrids was 10 mg.

The gonad from a healthy bird (no. Y77) 38 months old when killed still retained a small area of thickened germinal epithelium (fig. 10) which was proliferating secondary cords. Over most of the gonad, however, the epithelium had been reduced to a single layer of cells and a thin tunica developed beneath it (fig. 9). We have never found one of these abnormal gonads well encapsulated as is a normal adult testis. In the oldest of these gonads some of the largest cords become infiltrated with fat and typical fat-laden cells are often found. Although the presence of these cells may indicate degeneration we find no evidence that they disappear completely. We consider it rather remarkable that, despite clear evidence of some proliferative activity in these rudimentary and apparently non-growing adult gonads, there is so little evidence of actual degeneration.

By comparing figures 11 and 12 one can see the striking similarity between the primary and secondary cords of these

transformed ovaries and the young testicular tubules of a normal male dove. Larger cells with large deeply staining nuclei are found frequently and these resemble germ cells only because of their large size (fig. 13); we regard them as hypertrophied epithelial cells approaching degeneration. A few nuclei have been seen which are even three times as large as the one shown in figure 13. These giant cells seem to be formed mainly in old cords and not in newly-formed ones; this of course may mean that these cells are approaching degeneration, and degeneration of some of the cord cells accounts for the cellular debris sometimes found in the larger cords.

During all the stages described above a considerable amount of the wolffian body persists; in the oldest of the series the mesonephric tubules form a structure indistinguishable from an epididymis (fig. 14) and may even connect with the gonad. We thus find that all the materials essential to a functional male gonad are present, but their full development is never realized and this in turn may be basically associated with the fact that the testicular-like tubules would yield sterile products—if any products—due to hybridity. It may be noted that the minute right gonad can be identified histologically throughout all the stages of life; it undergoes atrophy quite as in normal doves and never forms tubules. Some intertubular tissue is of course observable (figs. 8, 9, 11, 13) in all of these mature abnormal gonads, but it exists only in small amount and there is little (see below) that we can declare concerning its nature or function. We have observed no well-differentiated sperm ducts, and we have failed to establish the presence or absence of traces of such ducts. The left oviduct remains thread-like and infantile throughout life (fig. 16)—twelve of these structures in adults had an average weight of 17 mg.—thus demonstrating that the associated abnormal gonad fails completely to secrete a detectable amount of ovarian hormone.

Supplementary data. In the case of hybrid Y77, aged 38 months, an attempt was made to stimulate one of these ab-

normal gonads with pituitary gonad-stimulating hormone (FSH + no. 474). Before dosage the bird was biopsied and the size of the gonad estimated; after observing its small rudimentary left gonad and a juvenile left oviduct the bird was injected daily for 11 days (4 mg./day) and sacrificed. No increase in size of either the gonad or infantile oviduct was evident or measurable, though large numbers of testicular-like elements were found. No real or marked difference could be declared, however, between this treated gonad (rather old hybrid) and other untreated ones from younger adult hybrids. At this stage of our study a lack of living hybrids prevented further experiments of this nature; but the ineffectiveness, or very slight effectiveness, of a large amount of gonad-stimulating hormone in this single instance provides evidence that these rudimentary gonads owe their undeveloped state not to absence or deficient production of gonad-stimulating hormone but to developmental restrictions associated with their hybrid origin. This conclusion is supported by repeated failure of good gonad-stimulating preparations to activate or enlarge the typical rudimentary right ovaries of doves and pigeons.

It is necessary to note here that a seemingly identical type of abnormal sexuality results from a nearly identical sub-family cross in which one parent is hybrid. It was noted above that crosses of *Zenaidura carolinensis* with *Zenaida vinaceorufa* appear to give fully fertile hybrids. One mating of such a male hybrid with a female *St. decaocta* gave, on macroscopic examination, offspring of the following sex types: thirteen males; fourteen with a single sex gland of the abnormal type we have described at length in this report; one without any macroscopic trace of a gonad; seven dead or killed embryos in which sex was not ascertained. It is further notable that this same cross was previously made (four matings) by Whitman ('19) who obtained twenty-six offspring for nine of which the sex was noted. Four of these nine were males, three were of 'doubtful' sex, and two had no gonads. These and other instances of the occurrence of 'no gonads' in hybrids and

other pigeons have been discussed elsewhere by Riddle ('25 a) who showed that pronounced masculine behavior could be observed in some of these cases, and who pointed out the probability that the complete absence of gonads in these cases followed the failure of primordial germ cells to reach and activate the anlage of the germinal ridge.

Each of the two above-mentioned fraternities supplied still another item of interest. One normally male hybrid reared by Whitman and examined by one of us was killed when quite healthy at the very advanced age of 15 years and 7 months. One individual of the fraternity reared by us was used in an attempted back-cross with a female of its paternal line, and in this connection we obtained specific records of its mating and sex behavior. At autopsy, while healthy and still stimulating its mate to lay eggs, this hybrid possessed a single left gonad and oviduct of the type described at length in this paper; but during the last 11 months of its life this bird copulated as a male, incubated eggs by day as does a male, was fully accepted as a male by a normal female mate, and thus proved that in its adult state it possessed the psyche of a male.

Other aspects of developmental repression in these sub-family hybrids and in still other pigeon-dove hybrids contribute to an understanding of the basis of the abnormal sexuality observed in these groups. Data concerning two items are presented in figure 1. (1) The testes of sub-family hybrids grow more slowly and attain a smaller adult size than is attained in either of the parent species—Mourning dove or Ring dove. The same is true for testis growth in hybrids from the still wider (family) cross of Common pigeon and Ring dove (see Riddle, '25 b). (2) Body size in the sub-family hybrids of both sex types, though quite variable, is significantly smaller than that of either sex of either of the parent species. Again, body size in the all-male hybrids from the family cross is somewhat higher (229 gm.) than that of Ring doves (165 gm.) but markedly less than that (334 gm.) of the type of Common pigeons used. The data for testis growth and for body size in Common pigeons and in Ring doves are taken from a previous

publication (Riddle, '28); the data of similar nature presented here in the form of curves for the two kinds of hybrids were hitherto unpublished. These data make it obvious that in hybrids from the widest possible crosses of pigeons there is not only repression or suppression of ovarian development, but likewise some developmental repression in testicular and somatic tissues as well.

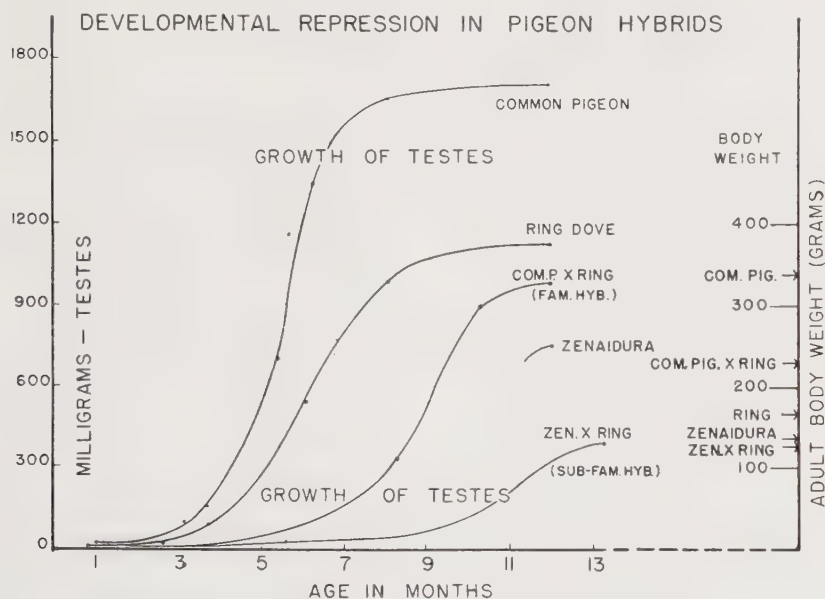


Fig. 1 Smoothed curves showing the rate of growth of testes in certain species of pigeons and in their normal male hybrids. Also, at right, comparison of adult body weight of certain hybrids from very wide crosses with those in the parental species.

DISCUSSION

That minor fraction of gonads of *Zenaidura* $\times$ *Streptopelia* hybrids which resemble ovaries at hatching, and which are wholly aberrant structures in adults, are shown by their histology to be immature retarded ovaries at hatching. As the bird matures these structures do not develop into functional ovaries—neither exocrine nor endocrine—but their slight further development is mainly or wholly in the direction of a

testis. In birds of from 1 to 3 years of age the left gonad is almost completely filled with structures resembling normal young testicular cords and, in some cases, even tubules with lumina. These gonads show an attempt at formation of true cortical cords of the second proliferation from the germinal epithelium, and later of testicular tubule-like elements from the medullary cord tissue. The result in the adult is a very abnormal, abortive and rudimentary gonad in which neither functional male nor female reproductive cells are formed. The persistence of considerable amounts of mesonephros in all cases, and the hypertrophy of some of the mesonephric tubules to form a structure quite like an epididymis in the oldest birds, further show that the trend in development is toward a male gonad. Much of this developmental miscarriage is doubtless associated with the aberrant chromosomal constitution of the cells of the germinal line as an incident of hybridity; but such an explanation applies with difficulty to the gonadless condition which is frequently encountered in these and other hybrids (Riddle, '25a) and it is important to recall that normal appearing testes, which are in fact plainly and wholly testicular in nature though moderately reduced in size, are readily formed in that much larger percentage of offspring which hatch with paired gonads (fig. 15).

The origin of the testis-like structures in this initially ovarian tissue is twofold—activated germinal epithelium and medullary cord cells. Cords derived from the former are homologous with the true cortical cords of a normal ovary, while those derived from the latter are homologous with true testicular tubules. Although these two types of cords become indistinguishable in older birds these left gonads of adults must be considered as containing a mixture of cords of primary and secondary origin. The individuals of this minority of total offspring which are merely retarded females at hatching are therefore birds which later undergo partial sex reversal.

The cords found and described in this study are very similar to those illustrated by Gray ('30) from the compensatory

right gonad of the female fowl following sinistral ovariectomy. There also the cords were found to have a double origin and it is stated that in the fowl the two kinds cannot be distinguished after they come to lie side by side in the medullary portion of the gonad. In the normal right rudimentary ovary of the fowl Brode ('28) likewise described structures derived from the medullary cords and homologous with testicular tubules which are similar to the ones described here. Crew and Fell ('22) found degeneration of testicular tubules in undescended testes of rabbit and cat, and their figures of early degenerating sterile tubules resemble the large cords found in the transformed ovaries of these sub-family hybrids. Thus these observations by others are in good accord with our interpretation of these structures as testicular-like elements. This interpretation is further supported by comparisons of these cords with young testicular cords found in hermaphrodite pigeons (Riddle and Schooley, unpublished data) whose gonads provide all gradations between young solid cords and sperm-producing tubules. It seems clear that these hybrid gonads make progress toward forming both cortical cords and seminiferous tubules.

In the particular group of sub-family hybrids especially reported upon here the early genetic impulse which initiates the development of an ovary in the embryo is overridden in late embryonic and post-natal life; this genetic impulse to ovarian development is suppressed early—but in a phase of the life cycle when this may be observed—and a subsequent slight development of testicular structures regularly occurs. This demonstration of gradual development in post-natal life of testicular elements in birds which hatch as females provides a significant new item concerning sexuality in birds. This phenomenon occurs in a very wide—but not the widest possible—cross of pigeons. In those widest crosses ovarian tissue is never or practically never developed, though testes which produce sperm of somewhat abnormal nature readily develop and some of these testes are accompanied by infantile left oviducts. These types or degrees of hybridity are

associated with two degrees of sex repression and in both cases still other aspects of developmental repression are found in both germinal and somatic tissues of the hybrids. In crosses of individuals belonging to different genera both sexes appear, though some restrictions upon fertility and a greater frequency of the gonadless condition and of hermaphroditism are observed than when intra-specific matings are involved. In inter-specific crosses fertility is often at its highest point and vigorous, fertile and long-lived offspring are obtained. In highly inbred strains fertility is again usually reduced and the bodily vigor of the race is also usually diminished. It thus appears that post-embryonic partial sex reversal and total exclusion of the female sex—both of which have now been observed in the offspring of very wide crosses of pigeons—are associated with one or another degree of widespread repression of general development which is observable in both the germinal and somatic tissues of both sexes.

SUMMARY

Crosses of male Mourning doves (*Zenaidura macroura carolinensis*) with female Ring doves (*Streptopelia decaocta*) have given hybrids of two sex types. First, an apparent excess of males (eighty-three cases) whose paired testes undergo slow but otherwise nearly normal development at all stages of life. Second, a well-represented minority (fifty-one cases) in which the nature of the gonad changes during post-natal life. At hatching the gonadal tissue is largely restricted to the left side; this tissue has the shape and appearance of ovary, its structure proves it to be an ovary already retarded in development, and it is accompanied by a single left oviduct. In later and adult stages the gland remains small and rudimentary; no clearly recognizable germ cells of either sex ever develop, the formation of primary and secondary sex cords continues, the gland becomes testis-like and filled with testicular-like tubules some of which have lumina, and an epididymis develops.

Other aspects of developmental repression in these and other hybrids from wide crosses of pigeons are examined and found to include the body weight of both sexes and the adult weights of the testes.

It thus appears that post-embryonic partial sex reversal and total exclusion of the female sex—both of which have been observed in the offspring of very wide crosses of pigeons—are associated with one or another degree of widespread repression of general development which is observable in both the germinal and somatic tissues of both sexes.

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EXPLANATION OF PLATES

All figures are unretouched photomicrographs. The magnifications given are actual, i.e., corrected for reductions. The preparation shown in figure 2 was fixed in Zenker's fluid followed by Mallory's triple stain. All others were fixed with Bouin's fluid and stained with hematein. Bird Y77 was given eleven daily injections of a gonad-stimulating pituitary extract.

PLATE 1

EXPLANATION OF FIGURES

- 2 Left gonad of *Zenaidura* $\times$ *Streptopelia* (sub-family) hybrid 3 days after hatching. Note the thickened germinal epithelium and one cord of cells of medullary origin deep within the medulla. Compare with a normal ovary shown in figure 3. $\times 200$.
- 3 Normal ovary of 8- to 10-day embryo of Ring dove showing many germ cells present in the cortex. $\times 200$.
- 4 Portion of medulla of left gonad from sub-family hybrid 15 days after hatching. A few cells showing resemblance to germ cells seen near the center of the photograph. Compare with figure 5. $\times 656$.
- 5 Portion of medulla of ovary from normal 8- to 10-day Ring dove embryo. Isolated germ cells presumably brought down by the first proliferation are shown near the lower center of the photograph. $\times 656$.
- 6 Portion of left gonad of sub-family hybrid no. 403, aged 5.3 months. Note clustering of cells from the germinal epithelium to form cords and also other cords lying in the medulla next to the thin tunica. $\times 656$.
- 7 Another region of the same gonad shown in figure 6. One large cord shown in cross section has its lumen filled with cellular debris. Also note the rather long cord near upper margin of figure which resembles a true testicular cord. $\times 656$.
- 8 Detailed view of cords in left gonad of sub-family hybrid no. S527, aged 10.8 months. $\times 656$.
- 9 Left gonad of sub-family hybrid no. Y77, aged 38 months. Note the extreme thinning of the germinal epithelium at the surface of the region shown. The entire gonad is made up of cords of varying size. $\times 200$.
- 10 Portion of same gonad as figure 9. Note that this part of the gonad surface, unlike that shown in figure 9, has a thickened germinal epithelium. $\times 200$.

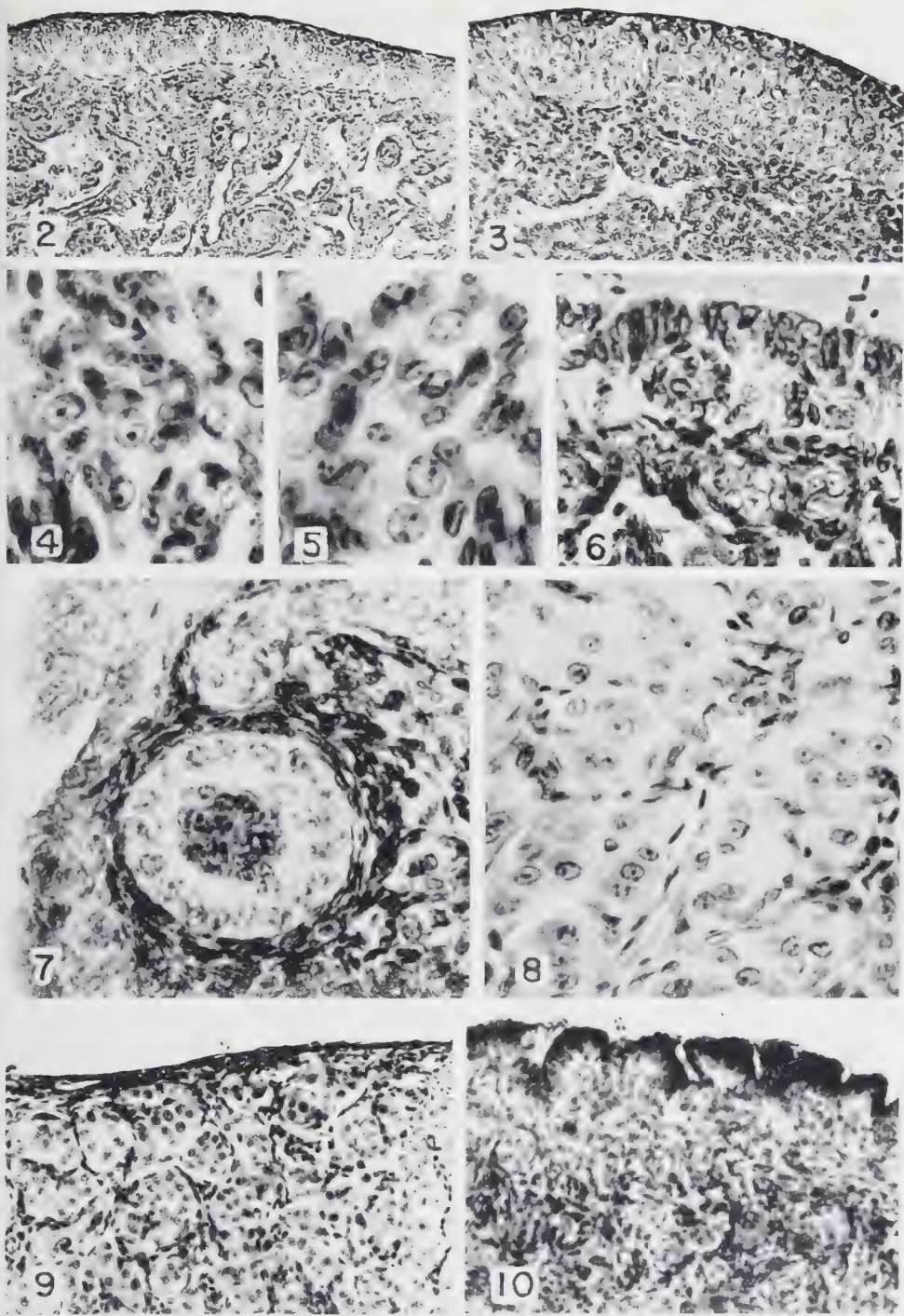
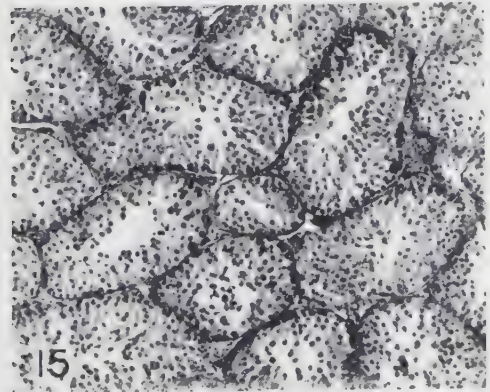
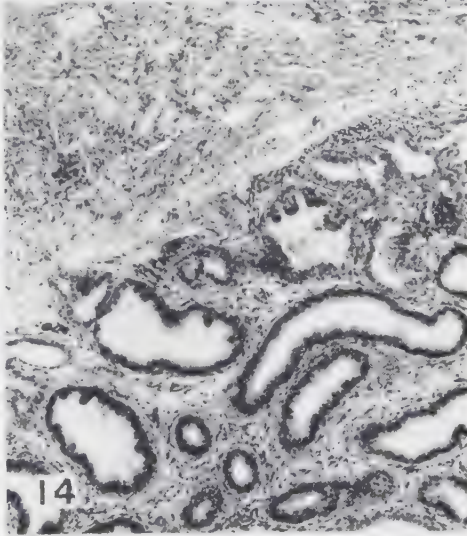
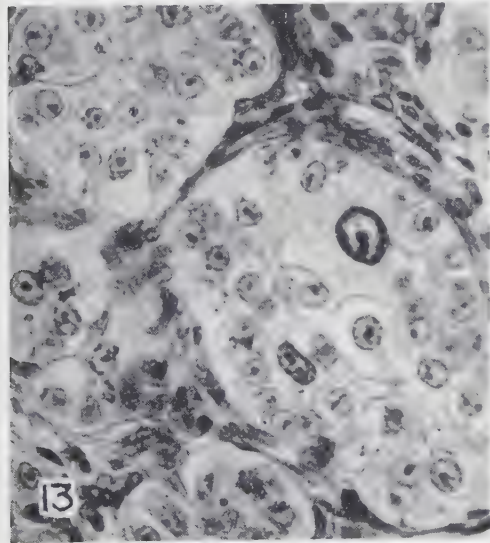
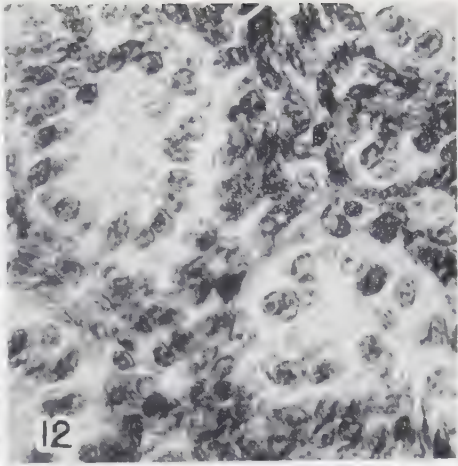
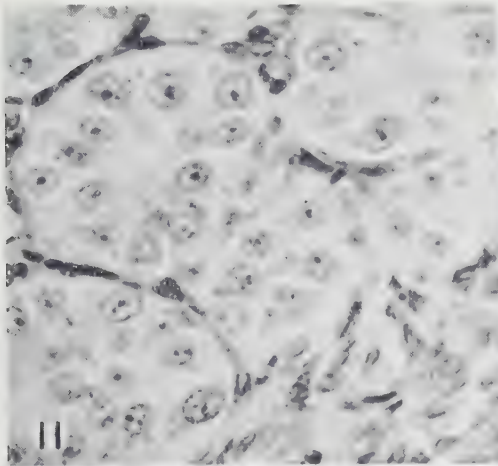


PLATE 2

EXPLANATION OF FIGURES

- 11 Portion of gonad from same bird as figure 8. Similarity of cords shown here to those of a normal immature testis can be seen by comparison with figure 12. $\times 656$.
- 12 Portion of testis from normal dove 13 days after hatching. $\times 656$.
- 13 Gonad from sub-family hybrid no. Y77. Note the giant cells in one of the large cords. $\times 656$.
- 14 Left gonad from sub-family hybrid no. Y77 at junction of epididymis with the gonad. $\times 88$.
- 15 Portion of testis of sub-family male hybrid (no. 534) dead of canker. $\times 88$.
- 16 Cross section of oviduct from sub-family hybrid no. Y77. $\times 66$.



THE BONE MARROW OF RATS MADE ANEMIC BY ADMINISTRATION OF SULFANILAMIDE

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Recently we reported the details of an anemia induced in rats by the daily oral administration of sulfanilamide (para-aminobenzenesulfonamide). The daily administration of 1 gm. of the drug per kilogram of body weight produced in 10 days a macrocytic type of an anemia characterized by marked reductions in the erythrocyte count, a corresponding reduction in the hematocrit determinations, an increase in the mean corpuscular volume and an increase in the percentage of reticulocytes in the peripheral blood. Toxic symptoms, such as cyanosis, gastric distention, irritability and occasional convulsions, were seen. This report is concerned with the changes observed in the bone marrow of rats in which changes in the peripheral blood, as indicated, were induced by administration of sulfanilamide.

Although there is a rapidly growing literature on both the clinical and experimental use of sulfanilamide, detailed studies on the effects of the drug on the cells of the bone marrow have not been reported. Much of the data contained in the reports of experimental studies have concerned the toxicity of the drug and the effects exerted upon the formed elements of the peripheral blood; there are only occasional references to the changes in the bone marrow.

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In studying lesions in white mice Hageman ('37) observed an increased incidence of eosinophils in the bone marrow preparations of animals which had been given sulfanilamide. Apparently no actual counts were made for no data regarding such counts were included in the report. Finklestone-Sayliss, Paine and Patrick ('37) deduced from studies made on the peripheral blood of animals which had been given sulfanilamide that there was marrow stimulation. The number of polymorphonuclear leukocytes was increased but there was no increase in the percentage of reticulocytes. No report was made of direct observations upon the bone marrow. Rimington and Hemmings ('38) gave daily doses of sulfanilamide to rats and observed a marked increase in the urinary and fecal excretion of porphyrin and a polyuria, but observations on the bone marrow were not reported. Kreutzmann and Carr ('38) gave prontosil to rabbits for 21 days. They observed an increase in the percentage of eosinophilic polymorphonuclear leukocytes in the peripheral blood and regarded this increase as indicative of depression of bone marrow. No statistical data were assembled. Wood ('38) made a numerical study of the cells in the bone marrow in a case in which acute hemolytic anemia developed during a course of sulfanilamide therapy. Marrow preparations of the sternum, vertebra and distal end of the femur were made. Cells were counted and a differential count was made. Wood deduced that there was a definite hyperplasia of the erythropoietic elements of the bone marrow.

PROCEDURE

Fifteen apparently healthy male rats of the Wistar strain were carefully selected. They ranged in weight from 200 to 250 gm., the average weight being 217 gm. The number of erythrocytes ranged from 8,400,000 to 11,100,000 (average 9,825,000) per cubic millimeter of blood, and the number of leukocytes ranged from 10,300 to 24,800 (average 17,850) per cubic millimeter of blood. Although there was a considerable variation in both the erythrocyte and leukocyte counts, for the purpose of this study we concluded that we had selected

a group of animals in which the bone marrow could well be accepted as normal.

Three of the fifteen animals were lightly anesthetized and killed by exsanguination. The femurs were dissected free and disarticulated, and the proximal portion was gently crushed in order to expose the marrow. Serial imprint preparations were made and then stained with the May-Grünwald Giemsa stain (Pappenheim's modification). A minimum of 500 cells were counted from each preparation and the percentage distribution of the erythroid and myeloid elements was computed. The data assembled from preparations made of the marrow of these three animals constituted the body of control data with which all the experimental data were contrasted.

The remaining twelve animals were given by mouth 1 gm. of sulfanilamide (para-aminobenzenesulfonamide) in 6 cc. of tap water daily in the morning. Two were lightly anesthetized and killed by exsanguination on the fourth day. Imprint preparations of the bone marrow were made in a manner identical to that described for the three control animals. Likewise, two animals were killed on the sixth day, two on the eighth, and one on each of the tenth, twelfth and fourteenth days. One animal died of toxic reactions during the experiment and two others were allowed to recover. Imprint preparations were made of the femoral marrow of all ten animals. One thousand cells were counted of each preparation and the percentage distributions of the cells were established.

RESULTS

For a description of the cells of both the myeloid and erythroid series occurring in the bone marrow of rats the reader is referred to the paper by Stasney and one of us (Higgins). It will be observed that a considerable shift in the percentages of the immature myeloid forms, from the data recorded, in 1935, has been tabulated in the control preparations of the current report. Twenty-four animals supplied the data from which the earlier report was prepared, whereas but three were killed to serve as a control for this study. Since the transition

from myeloblast to granulocyte is more or less a continuous one, it is conceivable that constancy in the percentage of any one stage of myeloid development would hardly maintain in different groups of animals. Thus differences in the actual percentages of the various stages are likely to occur. More significant is the fact that the total number of myeloid cells in this control group of three animals constituted 59.56% of all cells counted, while Stasney and one of us (Higgins) in a study of the marrow of twenty-four animals, found that the

TABLE 1
Percentage distribution of cells in the bone marrow

DAYS OF ADMINISTRATION OF SULFANILAMIDE							
	Control	4	6	8	10	12	14
Myeloblast	0.42	0.59	0.75	0.47	0.42	0.74	0.46
Leukoblast	2.83	1.89	1.29	1.28	0.84	0.74	0.57
Promyelocyte	2.31	6.29	3.22	2.04	3.64	2.97	5.72
Myelocyte	2.78	9.11	8.97	9.23	8.27	6.78	9.15
Metamyelocyte	21.08	17.88	15.68	14.12	19.07	13.28	11.89
Granulocyte	16.93	26.27	7.50	7.43	10.37	6.13	7.67
Eosinophils	11.75	3.64	4.53	2.97	2.66	5.94	6.06
Basophils	0.95	0.13	0.08	0.51	0.28	0.46	0.23
Normoblasts	36.51	30.07	56.30	60.49	52.31	61.61	57.55
Megakaryocyte	0.51	0.39	0.72	0.56	0.31	0.33	0.46
Plasma cells	0.97	1.05	0.46	0.27	0.42	0.27	0
Lymphocytes	2.96	2.69	0.50	0.63	1.40	0.74	0.23

myeloid cells constituted 62.75% of all cells counted. Furthermore, the percentage of erythroid cells, including both erythroblasts and normoblasts, constituted 36.51% of all cells tabulated from smears of marrow of these three control animals, while the data of Stasney and Higgins showed a mean erythroid percentage of 35.89.

The essential changes which have been observed in the smears of marrow of animals given sulfanilamide and killed at intervals during the administration of the drug have been condensed into tables 1 and 2. It seems evident from the data that even as early as 4 days changes occurred in the marrow which indicated an initial myeloid stimulation. Counts on the

formed elements of the peripheral blood of the two animals killed after 4 days of treatment showed a slight reduction in the erythrocyte count and a considerable decrease in the total number of leukocytes. This decrease in the number of leukocytes in the peripheral blood was reflected in the myeloid stimulation in the marrow, for an average relative increase of 6.63% in total myeloid cells was recorded for the two animals killed on the fourth day. The myeloid-erythroid ratio which had been established at 1.63:1.00 for our control group increased to 2.20:1.00 on the fourth day.

The myeloid stimulation which occurred in the bone marrow on the fourth day was clearly shown in the peripheral blood of the animals killed on the sixth day. The average leukocyte

TABLE 2
Myeloid-erythroid ratios in bone marrow

	DAYS OF ADMINISTRATION OF SULFANILAMIDE						
	Control	4	6	8	10	12	14
Total myeloid cells (per cent)	59.56	66.19	42.74	38.61	45.86	37.37	42.21
Total erythroid cells (per cent)	36.51	30.07	56.30	60.49	52.31	61.61	57.55
Myeloid-erythroid ratio	1.63:1	2.20:1	0.76:1	0.64:1	0.87:1	0.61:1	0.75:1

count made of the peripheral blood on two animals killed on the sixth day was 29,000 cells per cubic millimeter, while 6 days before, at the onset of the experiment, the mean leukocyte count of these two animals was 19,000. The mean erythrocyte count of the two animals killed on the sixth day was 7,400,000 cells per cubic millimeter which was a marked decline from the count of 10,200,000 recorded for these same animals before the administration of any drug. These changes in the peripheral blood were also reflected in the percentage distribution of cells in the marrow. As a result of the anemia there was a marked erythroid stimulation in the marrow resulting in a relative increase from 30.07% of all cells tabulated, to 56.30%. Whereas there was a decline in the relative

myeloid percentage from 66.19 to 42.74, on the sixth day, the conclusion is not indicated that there was any myeloid suppression. The marrow was exceedingly cellular and abundant and we are of the opinion that an absolute increase of both myeloid and erythroid elements occurred. Total counts of marrow cells have, of course, not been attempted. This rapid increase in erythroid components over and above the myeloid percentage had resulted in an inversion of the myeloid-erythroid ratio, which on the sixth day was 0.76:1.00.

The relations between the numbers of cells of the peripheral blood and those of the bone marrow of animals killed on the sixth day were all the more apparent in those animals killed on the eighth day of administration of the drug. At that time the peripheral blood contained an average of 31,000 leukocytes per cubic millimeter, while the average number of erythrocytes for the two animals was 5,900,000 per cubic millimeter. This further decline in the total number of erythrocytes, together with the increase in the total number of leukocytes was again reflected in the bone marrow by an even higher rise in the relative erythroid percentage coupled with a corresponding decline in the relative myeloid percentage. The data indicated that the anemia induced in the peripheral blood by the drug produced a marked stimulation of cells in the bone marrow.

The conditions which were observed on the eighth day were not essentially different in those animals killed on the tenth, twelfth and fourteenth days respectively. In all animals there was a greater relative increase in the erythroid elements, so that a myeloid-erythroid ratio of less than 1:00 was always found.

Although in the tabulations the eosinophilic leukocytes were not grouped into the eosinophilic myelocytes, metamyelocytes and mature eosinophils, there was no indication of any significant increase in the number of eosinophils in the marrow. Most of the cells were of the mature type with ring-shaped nuclei, but the relative percentages established for this cell

type did not indicate any eosinophilic stimulation, such as has been described hitherto.

One animal showed marked toxic reactions. These were first seen on the fourth day shortly after giving the drug, when the animal had definite convulsive symptoms. These reactions were more marked on the fifth day, and on the sixth day, when it appeared that the animal apparently would not recover, the rat was killed and the bone marrow was examined. Unfortunately red and white cell determinations on the peripheral blood were not made, but the bone marrow showed a marked relative erythroid suppression and a relative myeloid stimulation. The myeloid-erythroid ratio was nearly 4.00:1.00. Most of the erythroid cells were erythroblasts; very few normoblasts were seen. There were a large number of hypersegmented granulocytes in the smears, indicating perhaps some inhibition in the release of these cells into the blood stream.

In our early study on the peripheral blood of rats given sulfanilamide we observed the frequent appearance of granulocytes of the neutrophilic order, with a large number of lobes. Nuclei with as many as ten or twelve lobes were encountered. Smears of the marrow seem to indicate that these multilobular granulocytes begin to appear as early as the promyelocyte stage. Unusual and bizarre types of nuclear patterns were observed in certain promyelocytes. These continue to differentiate through the succeeding stages of myeloid development and give rise, we believe, to the hypersegmented granulocytes in the peripheral blood.

SUMMARY AND CONCLUSIONS

Bone marrow preparations of white rats which were given daily doses of sulfanilamide (1 gm. per kilogram of body weight) have been studied. Imprint preparations were made of femoral marrow removed from animals killed on the fourth, sixth, eighth, tenth, twelfth and fourteenth days respectively. The differential percentage distribution of the cells compris-

ing the marrow was established and these data were compared with those assembled from the marrow preparations of three control animals which had not received sulfanilamide. The following observations and deductions have been made.

1. On the basis of the relative distribution there is, after 4 days of administration of the drug, a greater myeloid stimulation than erythroid stimulation.

2. At 6 days, when anemia in the peripheral blood was seriously indicated, we observed that the erythroid stimulation was relatively greater than the myeloid stimulation.

3. The myeloid-erythroid ratio, whereas normally greater than one, was always less than one from the sixth day of the experiment to the end.

4. Eosinophilic stimulation in the bone marrow was not apparent.

5. The frequent appearance of multilobular granulocytes in smears of the peripheral blood of animals receiving sulfanilamide seems to be explained on the basis of the early modifications in the nuclear patterns of the promyelocytes and myelocytes.

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THE COMPARATIVE BEHAVIOR OF MAMMALIAN EGGS IN VIVO AND IN VITRO

VI. THE MATURATION OF HUMAN OVARIAN OVA

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TWO PLATES (SEVENTEEN FIGURES)

In an earlier paper in this series (Pincus and Enzmann, '35) it was shown that explantation alone led promptly to the first maturation division in cultured ovarian ova of the rabbit. In the rabbit the first maturation division normally occurs only after copulation (Pincus and Enzmann, '35, '37). Spontaneously ovulating mammals will exhibit this division regardless of copulation (Long and Mark, '11; Long and Evans, '22, et al.). Although woman is commonly recognized as spontaneously ovulating, it is remarkable that almost no ova in maturation division have been observed in human ovaries (Stieve, '26; Allen, Pratt, Newell and Bland, '30 a). The latter authors found only one ovum with a first polar body in over 200 eggs obtained from a large number of ovaries. It was considered a matter of some interest, therefore, to determine if human ova upon explantation would exhibit the same behavior as rabbit ova, i.e. to see whether a maturation potency inhibited in vivo would be expressed in vitro, and to ascertain whether the usual ovum activating treatments would accelerate maturation.

METHODS

Human ovaries obtained shortly after excision at operation were placed in Pannet-Compton or Tyrode solution at pH

7.0–7.2. Visible follicles were excised by carefully cutting away the surrounding tunica and interstitial tissue. In a number of instances the follicle diameters were measured. Into a small incision made in the follicle wall a pipette containing the balanced salt solution was inserted and the follicle was vigorously flushed, the expelled fluid being collected in a watch glass. Ordinarily after the first flushing, but sometimes not until the third or fourth the ovum was washed out surrounded in most instances by its cumulus (see figs. 1 and 5). Again in certain instances the ova were mounted on a slide in serum and their diameters measured.

All obviously atretic ova were discarded. These were identified by clear malformations of the vitellus such as partial collapse (fig. 1), elongation (fig. 2) or, occasionally, fragmentation (fig. 3).

Ordinarily one or more eggs from each set recovered from the ovaries were fixed at once in Bouin's fluid, sectioned and stained by the method described by Pincus ('39). The remaining ova were subjected to one of four treatments: 1) culture in human serum; 2) immersion for 15 to 30 minutes in a balanced salt solution containing cytolized human sperm; 3) exposure to a temperature of 46°C. for 3 minutes; 4) immersion in a 1.8% NaCl solution for 5 to 6 minutes. Immediately after treatment the eggs were washed once with serum and then cultured at 37°C. in small Carrel flasks containing 1 to 2 cc. of serum for the periods listed in table 1. Only ova sectioned and stained are listed in table 1. We consider proper cytological preparation of cultured ova imperative not merely because details obviously not observable in living ova are available, but also because often it is impossible, due to overlying cumulus cells, to identify polar bodies in these ovarian ova (contrast figs. 4 and 6).

RESULTS AND DISCUSSION

In a series of twenty follicles ranging in diameter from $3\frac{1}{2}$ to 18 mm. the diameters of the vitellus varied from 106 to 114 μ and the diameters of the zona pellucida from 117 to 128 μ ,

thus confirming Allen et al. ('30 a) in their statement that ova have attained full size in antra containing follicles (see Pincus, '36).

The data on 144 ova employed in this study are summarized in table 1. It will be noted that none of the thirty-four control ova fixed immediately after excision exhibited either chromosome spindles or polar bodies. All of them contained distinct nuclei with formed nuclear membranes located subcentrally in the vitellus (see fig. 7). In several nuclei pycnosis was evidently begun indicating that degenerative processes were occurring.

TABLE 1
The development of explanted ovarian ova

TREATMENT	HOURS CULTURED	TOTAL NUMBER OF EGGS	NUMBER OF EGGS WITH SPINDLES	NUMBER OF EGGS WITH POLAR BODIES	PER CENT OF EGGS ACTIVATED
(1)	(2)	(3)	(4)	(5)	(6)
None	0	34	0	0	0
None	8½	4	1	1	50
None	17 to 24	13	1	4	38
Sperm extract 15 to 30 min.	¼	5	0	0	0
Sperm extract 15 to 30 min.	1 to 2	10	3	0	30
Sperm extract 15 to 30 min.	15 to 48	40	8	10	45
Sperm extract 15 to 30 min. then to rabbit fallopian tubes	24 to 72	9	1	2	33
Heated to 46°C. for 3 min.	2	8	2	1	38
Heated to 46°C. for 3 min.	24 to 48	5	0	2	40
Hypertonic solution 5 to 6 min.	24 to 48	16	0	7	44

The most obvious feature of table 1 is that the percentage of ova activated is practically identical in all the series after 2 or more hours of culturing. Ova are considered activated if meiotic chromosomes are formed on a spindle or if a polar body has been extruded. It is notable that all ova that form meiotic chromosomes do not necessarily produce polar bodies. The initiation of the maturation division evidently requires something less than 2 hours. It is not necessarily carried to completion once initiated, as attested by the finding of arrested maturation spindles in ova cultured for the longer periods

(table 1, col. 4). It is barely possible that the heat and hypertonic solution treatments force the formation of polar bodies since all of the nine ova of these two series that did enter maturation and had been cultured for more than 2 hours, had formed polar bodies (table 1, col. 5), but the number of eggs involved is too small to permit generalization. Transplantation of the ova to rabbit fallopian tubes after exposure to sperm extracts does not apparently increase the percentage activated.

These data confirm previous findings with rabbits to the effect that removal of the ovum from the follicle is sufficient to initiate a nuclear maturation that would otherwise not occur. An average of about 40% of the ova in all series cultured for 2 hours or longer were activated. We believe that the 60% that was not activated consists largely of ova too far degenerated to be capable of activation (compare figs. 8 and 9). The obviously atretic condition of these eggs scarcely requires comment. In contrast the ova which have formed polar bodies (figs. 11, 12 and 14) exhibit the smooth, granular cytoplasm characteristic of functional ova, and this in spite of the fact that resorption of the zona pellucida is going on in both eggs. We believe, in fact, that the concurrence of atretic and normal processes accounts for the partial maturation of a number of eggs in this series. Thus the ovum of figure 15 began to form meiotic chromosomes but meiosis was arrested by atretic processes the effects of which are manifest in the hyaline cortex. In figure 16 we present an ovum in which the polar body lies beside a group of phagocytes. Similar phagocytosis has been demonstrated by Allen et al. ('30 a) in ova taken from the follicle and fixed at once, but never with accompanying polar body formation. Indeed one cultured ovum in our series (not sectioned and therefore not included in table 1) underwent cleavage and then fragmented (fig. 17). Subsequently, in such ova maturation and degeneration then proceed *pari passu*, and the extent of the former is limited by the degree of advancement of the latter.

The atretic processes are, in our opinion, probably not the result of the handling and culturing, but are initiated in the follicle and, if anything, delayed by explantation. We assert this because the maturation divisions of these ova are perfectly normal and presume that the rather high incidence observed must be due to the supervention of normal physiological conditions. Thus the chromosomes of both the polar body and the spindle of the ovum of figures 11 and 12 are distinct and easily counted. There are twenty-four chromosomes, typical diads (compare figs. 10 and 13), in each.

Why explanted ova enter into maturation so readily is not deducible from our data. We are inclined to believe that a positive inhibiting factor prevails *in vivo*. The ovum of figure 4 was obtained from a medium-sized follicle of an ovary kept in the refrigerator for 36 hours before the egg was removed. Unless we ascribe its formation to some stimulation by the cold we may deduce that the absence of some inhibitory factor carried by the circulation permitted polar body formation.

The ovulated human ovum contains the first polar body (Allen et al., '30 b; Lewis, '31; Pincus and Saunders, '37; Miller, Engel and Reimann, '38). It would seem therefore that mature, fertilizable human ova might be obtained simply by explantation of healthy ovarian eggs. This makes possible various interesting experimental approaches to the physiology and embryology of the human ovum.

We acknowledge with gratitude the kind cooperation of Doctor Pemberton, Dr. G. V. Smith, and Dr. John Rock and their associates of the Free Hospital for Women in making the specimens used in this study available to us.

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PLATE 1

EXPLANATION OF FIGURES

- 1 A collapsed atretic ovarian ovum as flushed from the follicle. Note the cumulus is still present.
- 2 An elongated atretic ovum.
- 3 A fragmented degenerate ovum.
- 4 Ovarian ovum with polar body. This polar body was formed in a medium sized follicle kept for 36 hours in the refrigerator. Note absence of cumulus cells and regular spherical shape of the vitellus.
- 5 A normal ovarian ovum freshly washed out of the ovarian follicle.
- 6 Ovum cultured for 24 hours after treatment with sperm extract. Note outgrowth of cumulus cells and polar body (at 2 o'clock).
- 7 Section of a control ovum showing thick cumulus and vesicular nucleus.
- 8 Section of an untreated ovum cultured for 17 hours. Note vacuolated cytoplasm and clumped chromatin, obvious signs of degeneration.

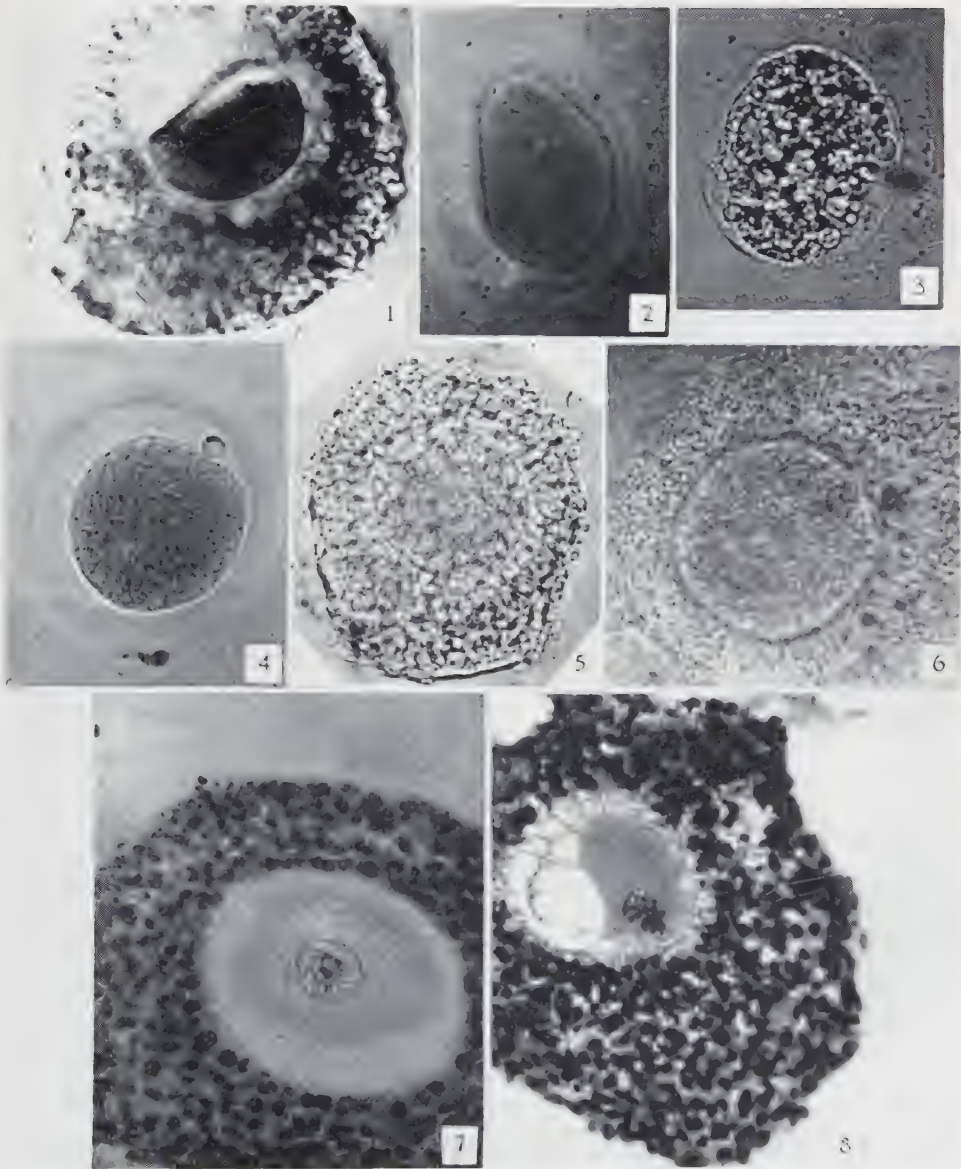


PLATE 2

EXPLANATION OF FIGURES

9 Section of an ovum treated with hypertonic solution and cultured for 48 hours. Note obvious degeneration of the cytoplasm and pycnotic nucleus.

10 Camera lucida drawings of the chromosomes of the spindle of the ovum of figure 12. Cut chromosomes are indicated by stippling.

11 Section of an ovum treated with hypertonic solution and cultured for 48 hours showing the polar body and below it to the left some of the egg chromosome. The spindle has disappeared. Note smooth cytoplasm.

12 Section of an ovum treated with hypertonic solution and cultured for 24 hours showing the second meiotic spindle and chromosomes. Note smooth cytoplasm.

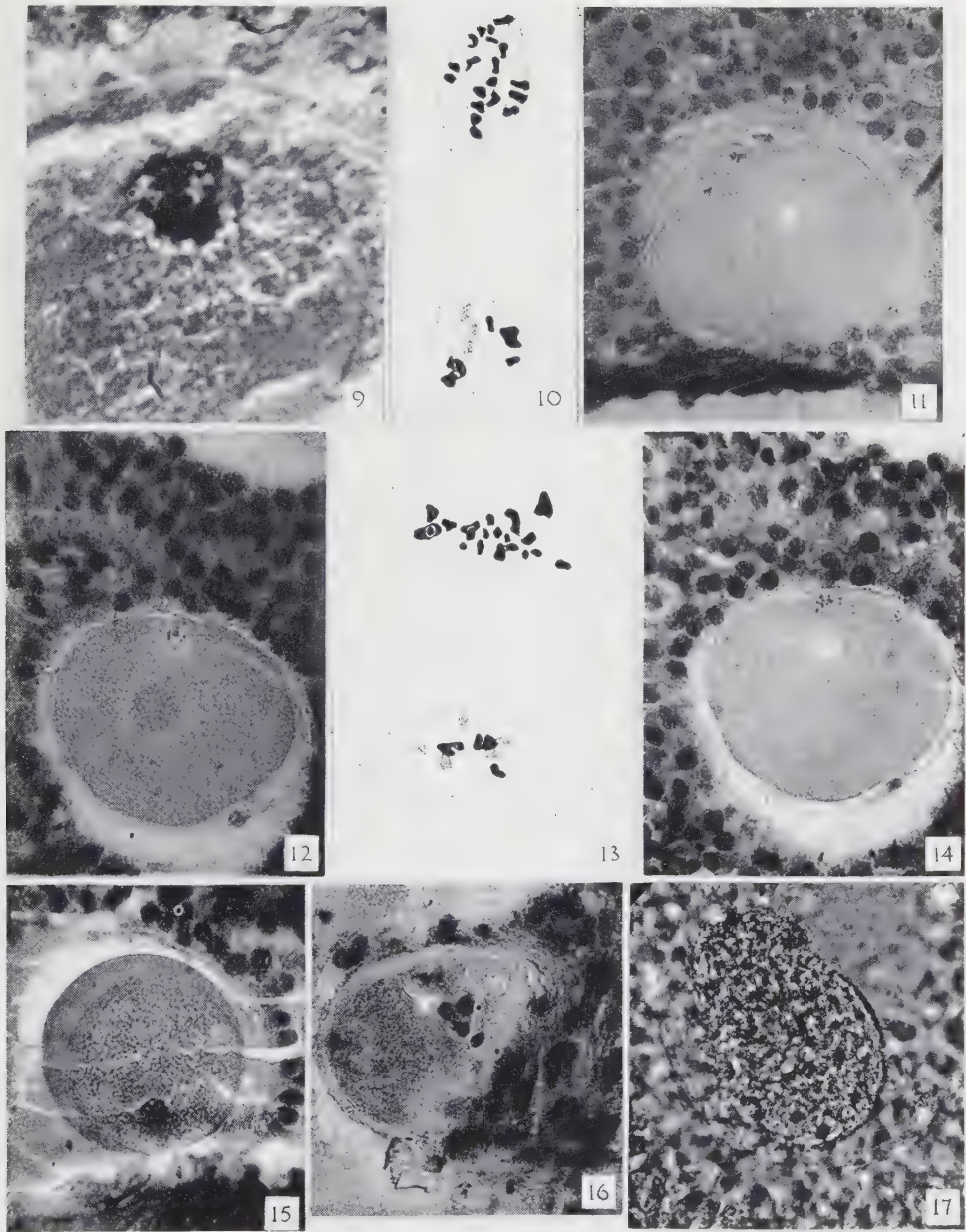
14 An adjacent section of the ovum of figure 12 showing some of the chromosomes of the polar body.

13 Camera lucida drawings of the chromosomes of the polar body of the ovum photographed in figures 12 and 14. The polar body was cut into two sections. Cut chromosomes are indicated by stippling. There are twenty-four chromosomes.

15 Section of an untreated ovum cultured for 24 hours. Meiotic chromosomes formed in the nucleus but no further development occurred. Note hyaline degenerating cortex.

16 Section of an egg exposed to sperm extract and cultured for 48 hours. Observe the polar body (at top) and immediately adjacent phagocytic cells.

17 Photograph of a cultured ovum 48 hours after treatment with hypertonic solution. Two blastomeres formed which subsequently degenerated.



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SUPPLEMENT

AMERICAN SOCIETY OF ZOOLOGISTS

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AMERICAN SOCIETY OF ZOOLOGISTS

CONSTITUTION

ARTICLE I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoologists."

Sec. 2. The object of the Society shall be the association of workers in the field of zoology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoological science.

ARTICLE II

MEMBERSHIP

Section 1. The membership of the Society shall consist of two classes:

a. Associate membership open to all actively engaged in the field of zoology who have had training in zoology equivalent to that required for the doctorate degree.

b. Active membership open to those who hold the doctor's degree in zoology or who have done work equivalent thereto, and who, in addition to the usual requirements for this degree, have published the results of substantial zoological research.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Associate members shall not hold office or vote, but may present papers before the Society without introduction. Associate members shall receive all literature issued by the Society, including the proceedings of the Society, and shall have the same privileges as active members in journal subscriptions.

Sec. 4. Associate members may be elected to active membership in the usual way when the research requirements of active membership have been met.

ARTICLE III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary, a Treasurer, and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer, and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices. (See by-laws.)

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled, until the next annual meeting, by appointment by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee consisting of three active members. It shall be the duty of this committee to nominate officers of the Society, including the permanent representatives which the Society is asked to name to various boards and councils. This committee shall make its nominations to the Secretary at least four months before the next annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society, the time and place of the annual meeting shall be determined by the Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

ARTICLE V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of the Executive Committee.

ARTICLE VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society upon approval of two-thirds of the members voting, subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

c. The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

d. Members unable to attend may submit their votes in writing to the Secretary.

BY-LAWS

ARTICLE I

DUES

Section 1. The annual dues for active and associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

Sec. 2. Any member in arrears of dues for two years should be dropped by the Executive Committee. Upon payment of arrearage he shall be reinstated.

ARTICLE II

PRESIDENT

Section 1. The President shall preside at scientific sessions and at the business meetings.

Sec. 2. He shall also appoint the committees prescribed by the constitution and by-laws.

ARTICLE III

VICE-PRESIDENT

Section 1. The Vice-President shall preside at sessions designated by the President.

Sec. 2. He shall also assume the duties of the President in the latter's absence.

ARTICLE IV

SECRETARY

Section 1. The Secretary shall keep the records of the Society.

Sec. 2. He shall be responsible for all arrangements for the meetings, including the appointment of local committees.

Sec. 3. Whenever the proper officers of a number of related societies shall have a conference covering matters of mutual interest to the several societies, he shall act as the delegate or representative of this Society.

Sec. 4. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society, and

Sec. 5. He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

ARTICLE V

TREASURER

Section 1. The Treasurer shall be in charge of the funds of the Society.

Sec. 2. At the annual business meeting of the Society he shall present a statement to date of the funds of the Society.

Sec. 3. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society.

ARTICLE VI

AUDITING COMMITTEE

The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

ARTICLE VII

PROGRAM RULES

In matters relating to programs for annual meetings, the following rules shall be observed:

Section 1. Titles and abstracts of papers to be presented by any method at the annual meeting must be sent to the Secretary in duplicate before the final date set by him. The length, form, and arrangement of the abstracts must comply with the requests of the Secretary in order to insure prompt publication by The Wistar Institute. The titles and abstracts of papers which do not conform to such requests or papers which are received late may be presented by title only.

Sec. 2. Titles shall be listed in groups according to subject matter, the groups to be determined by the Secretary.

Sec. 3. Whenever conditions require, the Secretary shall schedule two or more groups for the same hour and arrange the program to bring together papers on subjects of more general interest for meetings of the whole Society.

Sec. 4. Each member may have not more than fifteen minutes of the time of the Society at the annual meeting. This time may be used by him to present one or more papers personally or to introduce papers of non-members.

Sec. 5. All papers not read when called for as listed shall be placed at the end of the group list, and if not read when called for the second time, shall be read by title only.

Sec. 6. The titles of 'introduced' papers may be listed in the groups after the titles of papers to be read by members.

LIST OF FORMER OFFICERS

AMERICAN MORPHOLOGICAL SOCIETY

<i>President</i>	<i>Vice-President</i>	<i>Secretary-Treasurer</i>
1890—E. B. Wilson		J. P. McMurrich
1891—C. O. Whitman	E. L. Mark	J. P. McMurrich
1892—C. O. Whitman	H. F. Osborn	J. P. McMurrich
1893—C. O. Whitman	E. B. Wilson	J. P. McMurrich
1894—C. O. Whitman	W. B. Scott	G. H. Parker
1895—E. B. Wilson	W. B. Scott	G. H. Parker
1896—E. L. Mark	H. F. Osborn	G. H. Parker
1897—C. S. Minot	S. I. Smith	G. H. Parker
1898—H. F. Osborn	T. H. Morgan	G. H. Parker
1899—E. G. Conklin	W. M. Wheeler	Bashford Dean
1900—T. H. Morgan	H. C. Bumpus	J. S. Kingsley
1901—J. S. Kingsley	E. A. Andrews	T. H. Montgomery
1902—H. C. Bumpus	G. H. Parker	M. M. Metcalf

Additional Members of the Executive Committee

1891—E. B. Wilson	1897—J. S. Kingsley
H. F. Osborn	Bashford Dean
1892—E. L. Mark	1898—C. B. Davenport
T. H. Morgan	F. R. Lillie
1893—T. H. Morgan	1899—J. P. McMurrich
C. B. Davenport	G. H. Parker
1894—E. A. Andrews	1900—F. R. Lillie
F. H. Herrick	Jacob Reighard
1895—T. H. Morgan	1901—C. F. W. McClure
S. Watase	C. W. Hargitt
1896—E. G. Conklin	1902—H. S. Jennings
William Patten	R. G. Harrison

AMERICAN SOCIETY OF ZOOLOGISTS

<i>EASTERN BRANCH</i>	<i>President</i>	<i>CENTRAL BRANCH</i>
G. H. Parker	1903	Jacob Reighard
E. A. Andrews	1904	C. H. Eigenmann
W. E. Castle	1905	F. R. Lillie
W. E. Castle	1906	C. C. Nutting
C. B. Davenport	1907	S. A. Forbes
W. M. Wheeler	1908	E. A. Birge
H. S. Jennings	1909	E. A. Birge
T. H. Montgomery	1910	C. E. McClung
H. V. Wilson	1911	George Lefevre
A. G. Mayer	1912	H. B. Ward
Raymond Pearl	1913	H. B. Ward

LIST OF FORMER OFFICERS

EASTERN BRANCH

Jacob Reighard
W. E. Castle
William Patten
William Patten
F. H. Herrick
H. S. Jennings
H. V. Wilson
H. H. Wilder
H. E. Crampton
G. A. Drew
Alex. Petrunkevitch

Vice-President

1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913

CENTRAL BRANCH

H. F. Nachtrieb
S. J. Holmes
William A. Loey
George Lefevre
H. B. Ward
M. F. Guyer
M. F. Guyer
H. F. Nachtrieb
R. H. Walcott
C. M. Child
C. M. Child

Secretary-Treasurer

G. A. Drew
G. A. Drew
H. S. Pratt
H. S. Pratt
C. J. Herrick
L. L. Woodruff
L. L. Woodruff
H. W. Rand
Raymond Pearl
J. H. Gerould
Caswell Grave

1903
1904
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1908
1909
1910
1911
1912
1913

Frank Smith
F. R. Lillie
C. E. McClung
T. G. Lee
T. G. Lee
T. G. Lee
Charles Zeleny
H. V. Neal
H. V. Neal
W. C. Curtis
W. C. Curtis

Executive Committeemen

F. R. Lillie
T. H. Montgomery
H. C. Bumpus
H. S. Jennings
E. A. Andrews
W. R. Coe
G. A. Drew
M. M. Metcalf
D. H. Tennent
R. G. Harrison
H. E. Jordan
C. E. McClung
George Lefevre
T. G. Lee

Herbert Osborn
C. H. Eigenmann
J. G. Needham
S. J. Holmes
W. A. Loey
C. M. Child
R. H. Walcott
W. C. Curtis
Oscar Riddle
H. B. Ward
Chauncey Juday
H. W. Norris
C. E. McClung
H. F. Nachtrieb

AMERICAN SOCIETY OF ZOOLOGISTS (AMALGAMATED)

	<i>President</i>	<i>Vice-President</i>	<i>Executive Committeemen</i>
1914.	C. E. McClung	M. F. Guyer	H. E. Jordan—1 year
1915.	W. A. Locy	W. E. Ritter	H. F. Nachtrieb—2 years
1916.	D. H. Tennent	Charles Zeleny	H. V. Wilson—3 years
1917.	M. M. Metcalf	Charles Zeleny	George Lefevre—4 years
1918.	George Lefevre	L. L. Woodruff	A. F. Shull—5 years
1919.	C. M. Child	H. H. Wilder	D. H. Tennent—5 years
1920.	Gilman A. Drew	Caswell Grave	R. P. Bigelow—5 years
1921.	Charles A. Kofoid	Aaron T. Treadwell	L. J. Cole—4 years
1922.	H. H. Wilder	Bennet M. Allen	H. V. Wilson—5 years
1923.	M. F. Guyer	Robert A. Budington	M. M. Metcalf—5 years
1924.	R. G. Harrison	R. K. Nabours	George Lefevre—5 years
1925.	C. R. Stockard	C. R. Moore	C. M. Child—5 years
1926.	S. O. Mast	W. C. Allee	G. A. Drew—5 years
1927.	S. J. Holmes	Robert Chambers, Jr.	C. A. Kofoid—5 years
1928.	Caswell Grave	M. H. Jacobs	H. H. Wilder—5 years
1929.	C. B. Davenport	Fernandus Payne	J. H. Gerould—1 year
1930.	H. V. Neal	E. E. Just	M. F. Guyer—5 years
1931.	Fernandus Payne	D. E. Minnich	R. G. Harrison—5 years
1932.	W. C. Curtis	L. V. Heilbrunn	C. R. Stockard—5 years
1933.	Charles Zeleny	J. H. Bodine	S. O. Mast—5 years
1934.	A. H. Sturtevant	H. W. Rand	S. J. Holmes—5 years
1935.	R. W. Hegner	Sewall Wright	Caswell Grave—5 years
1936.	W. C. Allee	C. W. Metz	C. B. Davenport—4 years
1937.	F. L. Hisaw	Helen D. King	H. V. Neal—5 years
1938.	M. H. Jacobs	T. S. Painter	Fernandus Payne—5 years
			W. C. Curtis—5 years
			Charles Zeleny—5 years

Secretary-Treasurer

1914–1918.	Caswell Grave	1918–1921.	W. C. Allee
	<i>Secretary</i>		<i>Treasurer</i>
1921–1924.	W. C. Allee	1922.	D. H. Tennent
1925–1928.	D. E. Minnich	1923–1924.	R. H. Bowen
1928–1929.	L. B. Arey	1925–1930.	L. B. Arey
1929–1930.	D. E. Minnich	1930–1933.	A. B. Dawson
1930–1933.	W. H. Cole	1933–1935.	B. H. Willier
1933–1936.	H. B. Goodrich	1935–1938.	W. N. Hess
1936–1939.	E. G. Butler		

*Representative in the Division of Biology and Agriculture,
National Research Council*

M. F. Guyer 1919–1921	H. S. Jennings 1922–1925	H. S. Jennings 1929–1931
William Patten 1921–1924	L. L. Woodruff 1925–1928	L. C. Dunn 1931–1932
J. T. Patterson 1924–1927	E. G. Conklin 1926–1929	J. H. Bodine 1932–1935
G. H. Parker 1926–1929	F. R. Lillie 1919–1923	L. V. Heilbrunn 1935–1938
G. H. Parker 1919–1922	E. G. Conklin 1923–1926	

Associate Editors of the Journal of Morphology

1921.	Gary N. Calkins	J. S. Kingsley	William Patten
1921-1922.	E. G. Conklin	M. F. Guyer	W. M. Wheeler
1921-1923.	C. A. Kofoed	F. R. Lillie	J. T. Patterson
1922-1924.	G. A. Drew	H. V. Neal	L. L. Woodruff
1923-1925.	Caswell Grave	D. H. Tennent	H. V. Wilson
1924-1926.	Helen Dean King	S. O. Mast	A. A. Schaeffer
1925-1927.	R. W. Hegner	Fernandus Payne	H. B. Ward
1926-1928.	L. J. Cole	J. H. Gerould	M. H. Jacobs
1927-1929.	W. C. Curtis	J. Percy Moore	A. F. Shull
1928-1930.	Edgar Allen	G. A. Baitsell	R. E. Coker
1929-1931.	S. R. Detwiler	R. S. Lillie	H. J. Muller
1930-1932.	W. R. Coe	E. N. Harvey	H. E. Jordan
1931-1933.	L. V. Heilbrunn	S. O. Mast	G. K. Noble
1932-1934.	D. E. Minnich	B. H. Willier	L. L. Woodruff
1933-1935.	L. R. Cleveland	F. L. Hisaw	Franz Schrader
1934-1936.	Charles Zeleny	J. F. Daniel	J. S. Nicholas
1935-1937.	D. H. Tennent	L. C. Dunn	C. G. Hartman
1936-1938.	Sally Hughes-Schrader	E. E. Just	D. H. Wenrich

LIST OF PLACES OF MEETING

AMERICAN MORPHOLOGICAL SOCIETY

1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

CENTRAL NATURALISTS

1899—Chicago	1900—Chicago
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SOCIETY OF AMERICAN ZOOLOGISTS

1901—Chicago	1902—Washington
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AMERICAN SOCIETY OF ZOOLOGISTS

EASTERN BRANCH	JOINT MEETINGS	CENTRAL BRANCH
1903—Philadelphia	1905—Ann Arbor	1903—St. Louis
1904—Philadelphia	1908—Baltimore	1905—(Mch.) Chicago
1906—New York	1911—Princeton	1907—(Mch.) Madison
1907—New Haven	1912—Cleveland	1907—Chicago
1909—Boston	1913—Philadelphia	1910—(Apr.) Iowa City
1910—Ithaca		1910—Minneapolis
		1912—(Apr.) Urbana

MEETING PLACES

1914—Philadelphia	1922—Boston	1930—Cleveland
1915—Columbus	1923—Cincinnati	1931—New Orleans
1916—New York	1924—Washington	1932—Atlantic City
1917—Minneapolis	1925—New Haven	1933—Boston
1918—Baltimore	1926—Philadelphia	1934—Pittsburgh
1919—St. Louis	1927—Nashville	1935—Princeton
1920—Chicago	1928—New York	1936—Atlantic City
1921—Toronto	1929—Des Moines	1937—Indianapolis
		1938—Richmond

PROGRAM FOR THE THIRTY-SEVENTH ANNUAL
MEETING OF THE AMERICAN SOCIETY
OF ZOOLOGISTS

OHIO STATE UNIVERSITY

COLUMBUS, OHIO

DECEMBER 28, 29, 30, 1939

The Society will meet in conjunction with Section F of the American Association for the Advancement of Science and in association with several other biological societies. All meetings for the presentation of papers and demonstrations will be held in buildings on the campus of Ohio State University.

As features of the meeting the following special sessions have been arranged. A symposium on 'Speciation,' organized by Dr. Th. Dobzhansky, will be held jointly with the Genetics Society of America. Dr. M. H. Jacobs will lead a symposium on 'Experimental Study of Cellular Organization.' In addition, the Society will join with the American Society of Naturalists in sponsoring a symposium on 'Defense Mechanisms in Plants and Animals' arranged by Dr. W. H. Taliaferro.

The Biologists' Smoker will be held on the first evening of the meeting, Thursday, December 28th at 9:30 o'clock at the Neil House.

The Annual Dinner of the American Society of Zoologists, to which all zoologists and friends are invited, will be held at 6:30 o'clock Friday evening, December 29th at the Deshler-Wallick Hotel. Dr. Wesley R. Coe, vice-president of Section F of the American Association for the Advancement of Science, will give an address on 'Divergent Pathways in Functional Development.'

The Secretary has received no notification of special reduction in railway fares for the Columbus meeting. Members are advised, however, to consult their local railway ticket agents in regard to possible convention or holiday rates.

LOCATION OF MEETINGS

Hamilton Hall, in which all sessions will be held, with the exception of the Symposium on Thursday afternoon, is situated on Neil Avenue at one corner of the Ohio State University Campus. Trolley cars marked 'Neil Avenue,' which run directly to Hamilton Hall, can be obtained in downtown Columbus at the corner of Broad and Front Streets, near the Deshler-Wallick Hotel.

University Hall, in which the joint symposium with the Genetics Society will be held on Thursday afternoon, is situated on the University campus a short walk from Hamilton Hall.

The Deshler-Wallick Hotel, which will serve as hotel headquarters for the Society, is located in downtown Columbus seven short blocks from the Union Station. The hotel is about 3 miles from the Ohio State University Campus.

DINNER TICKETS

Tickets for the Zoologists' Dinner on Friday evening will be on sale at the Information Desk in Hamilton Hall. In order to assist the local committee in making arrangements, all persons planning to attend the dinner are urged to purchase tickets as early as possible.

LUNCHEONS

No arrangements for special biologists' luncheons have been made this year. However, on the University campus within easy walking distance of Hamilton Hall are two cafeterias, Pomerene Refectory and the Ohio Union. Several other restaurants are situated in close proximity to Hamilton Hall.

PROGRAM

THURSDAY, DECEMBER 28TH

- 9:30 A.M. Section A. *Embryology*. Papers 1 to 11. Room 211, Hamilton Hall.
Section B. *Cellular Physiology*. Papers 12 to 27. Room 312, Hamilton Hall.
Section C. *General Physiology*. Papers 28 to 42. Room 416, Hamilton Hall.
Section D. *Endocrinology*. Papers 43 to 52. Room 122, Hamilton Hall.
- 2:00 P.M. Joint Session with the Genetics Society of America. Chapel, University Hall
Symposium on '*Speciation*,' Th. Dobzhansky, presiding.
1. Breeding structure of populations in relation to speciation. Sewall Wright, The University of Chicago.
2. Speciation problems in birds. E. M. Mayr, American Museum of Natural History.
3. Speciation in *Peromyscus*. L. R. Dice, University of Michigan.
4. Levels of divergence in *Drosophila* speciation. Warren P. Spencer, College of Wooster
5. Speciation as a stage in the evolutionary divergence. Th. Dobzhansky, California Institute of Technology.
- 9:30 P.M. *Biologists' Smoker*. Neil House.

FRIDAY, DECEMBER 29TH

- 9:30 A.M. Section A. Symposium on '*Experimental Study of Cellular Organization*,' M. H. Jacobs, presiding. Program on page 19.
Room 122, Hamilton Hall.
Section B. *General Physiology*. Papers 55 to 69. Room 211, Hamilton Hall.
Section C. *Embryology*. Papers 70 to 82. Room 416, Hamilton Hall.
Section D. *Endocrinology*. Papers 83 to 94. Room 312, Hamilton Hall.
- 12:00 M. Annual Business Meeting of Section F, A.A.A.S., Room 122, Hamilton Hall.
- 12:10 P.M. Annual Business Meeting of the American Society of Zoologists, Room 122, Hamilton Hall.
- 2:00 P.M. *Demonstration Program*. Papers 151 to 182. Rooms 401 and 411, Hamilton Hall. (Motion pictures in Room 312.) See page 22.
- 6:30 P.M. *Zoologists' Dinner*. Address by Dr. Wesley R. Coe, vice-president of Section F, A.A.A.S., on '*Divergent Pathways in Functional Development*.' Hall of Mirrors, Deshler-Wallick Hotel.

SATURDAY, DECEMBER 30TH

- 9:30 A.M. Section A. *General Physiology*. Papers 95 to 110. Room 122, Hamilton Hall.
Section B. *Protozoology*. Papers 111 to 124. Room 211, Hamilton Hall.
Section C. *Cytology; Histology; Vertebrate Morphology*. Papers 125 to 139. Room 312, Hamilton Hall.
Section D. *Ecology; Parasitology; Miscellaneous*. Papers 140 to 150. Room 416, Hamilton Hall.
- 2:00 P.M. Joint Session with the American Society of Naturalists. Chapel, University Hall.
Symposium on '*Defense Mechanisms in Plants and Animals*,' W. H. Taliaferro, presiding.
1. Local reactions in plants. F. W. Went, California Institute of Technology.
 2. Generalized reactions in plants. W. C. Price, Rockefeller Institute for Medical Research.
 3. Local and generalized reactions in animals. William Bloom, The University of Chicago.

The Annual Dinner of the American Society of Naturalists will be held on Saturday evening, December 30th, 6:30 P.M. at the Deshler-Wallick Hotel. The presidential address will be given by Prof. I. F. Lewis, University of Virginia, on 'Cell Reactions.'

LIST OF TITLES

THURSDAY MORNING SESSION, DECEMBER 28TH

Hamilton Hall, Ohio State University

This session will be held in four sections, as shown below.

Section A. *Embryology*, 9:30 A.M.; Room 211, Hamilton Hall

1. The effect of some inhibitors of carbohydrate metabolism on the development of the frog egg. (8 min.) (Lantern.) C. M. Pomerat and R. Haringa, Clark University and the University of Alabama.
2. The capsular fluid of *Amblystoma punctatum* eggs. (10 min.) (Lantern.) Oscar W. Richards, Research Department, Spencer Lens Co.
3. The potency of the isolated vegetal hemisphere (presumptive endoderm) of the blastula of *Amblystoma punctatum*. (12 min.) (Lantern.) Louis T. Stableford, Yale University. (Introduced by J. S. Nicholas.)
4. The alteration of developmental pattern in sand dollar eggs with pilocarpine. (15 min.) (Lantern.) Olin Rulon, Wayne University.
5. A comparison of the development of *Arbacia* half-eggs activated before and after centrifugation. (15 min.) (Lantern.) Ethel Browne Harvey, Princeton University.
6. Evidence of role of sulphhydryl group in phenomena of fertilization. (10 min.) (Lantern.) Grace Townsend, Marine Biological Laboratory. (Introduced by Ralph Lillie.)
7. The effect of glutathione on sensitivity of *Arbacia* germ cells to x-ray considered from the viewpoint of Child's gradient theory. (5 min.) (Lantern.) Grace Townsend, Marine Biological Laboratory. (Introduced by Ralph Lillie.)
8. The effect of colchicine on cytoplasm and nuclei of the zebra fish blastomeres. (15 min.) (Lantern.) Edward C. Roosen-Runge, Brown University.
9. Experiments on polarity determination in *Tubularia regenerates*. (15 min.) (Lantern.) James A. Miller, University of Michigan.
10. Some processes of development in the posterior end of the lamprey embryo analyzed by localized vital staining. (15 min.) (Lantern.) Richard Weissenberg, The Wistar Institute. (Introduced by Clyde E. Keeler.)
11. Development of the network of the adepidermal melanophores in the tadpoles of *Discoglossus pictus*. (12 min.) (Epidiascope.) Hans Elias. (Introduced by Frederic T. Lewis.)

Section B. *Cellular Physiology*, 9:30 A.M.; Room 312, Hamilton Hall

12. The effect of calcium on the physical properties of the endothelial cement. (5 min.) (Lantern.) Robert Chambers and B. W. Zweifach, Laboratory of Cellular Physiology, New York University.
13. Alterations in the physical properties of the red cell. (10 min.) (Lantern.) Benjamin W. Zweifach, Laboratory of Cellular Physiology, New York University. (Introduced by M. J. Kopac.)
14. Chemical and structural features of the surface of red cells and ghosts. (5 min.) (Lantern.) A. K. Parpart and A. J. Dziemian, Princeton University.
15. The action of lipase on the cell surface. (10 min.) (Lantern.) R. Ballentine and A. K. Parpart, Princeton University.
16. The effect of temperature on cell permeability and on cell respiration. (10 min.) (Lantern.) F. R. Hunter, Rhode Island State College.
17. Quantum requirements for photodynamic hemolysis. (15 min.) (Lantern.) Harold F. Blum, National Cancer Institute, United States Public Health Service, and Howard W. Gilbert, Washington Biophysical Institute. (Introduced by C. B. Davenport.)
18. Spiral movement, rhythms and other characteristics of leucocytic behavior. (15 min.) (Lantern; motion picture.) A. A. Schaeffer, Temple University.
19. The stimulating action of citrates and oxalates on the Nereis egg. (15 min.) (Lantern.) Karl M. Wilbur, University of Pennsylvania. (Introduced by L. V. Heilbrunn.)
20. Viscosity and gelation in the Arbacia egg. (6 min.) (Lantern.) Robert Chambers, Laboratory of Cellular Physiology, New York University.
21. The suspension of activity in the fertilized Arbacia egg by early immersion in KCl. (4 min.) (Lantern.) E. L. Chambers and Robert Chambers, Laboratory of Cellular Physiology, New York University.
22. Factors affecting rate of cleavage in Arbacia; a preliminary study of homo-typic extracts. (15 min.) (Lantern.) W. C. Allee and Asher J. Finkel, The University of Chicago.
23. The effect of monochromatic ultraviolet radiation on the eggs of certain invertebrates. (10 min.) (Lantern.) Alexander Hollaender, Myrna F. Jones and Leon Jacobs, Divisions of Industrial Hygiene and Zoology, National Institute of Health. (Introduced by Mary Juhn.)
24. Effects of temperature on the radiosensitivity of cells. (8 min.) (Lantern.) T. C. Evans, State University of Iowa.
25. X-ray evidence for the reality of virus particles of large size. (15 min.) (Lantern.) John W. Gowen, Iowa State College.
26. The significance of the 'flash' of luminescence following anaerobiosis of luminous bacteria. (15 min.) (Lantern.) Frank H. Johnson and K. L. van Schouwenburg, Princeton University and the Biophysical Research Group, Utrecht, Holland.
27. Changes in the absorption spectrum of luciferin solutions. (15 min.) (Lantern.) Aurin M. Chase, Princeton University. (Introduced by E. N. Harvey.)

Section C. *General Physiology*, 9:30 A.M.; Room 416, Hamilton Hall

28. Reversible depression of the protoplasmic E.M.F. of *Valonia* by cyanide and ether. (15 min.) (Lantern.) Gordon Marsh, State University of Iowa. (Introduced by Eleanor H. Slifer.)
29. The electrical potential of frog skin. (8 min.) (Lantern.) T. Cunliffe Barnes, W. J. Coe and H. L. Golubock, Yale University.
30. Antagonism between heavy water and adrenalin on the rate and action current of the heart of the turtle. (7 min.) (Lantern.) T. Cunliffe Barnes, Yale University.
31. The relation between 'spontaneous' activity and synaptic transmission in the nervous system of the crayfish. (15 min.) (Lantern.) C. Ladd Prosser, University of Illinois.
32. The influence of potassium on the sensitivity of intestinal muscle to acetylcholine. (10 min.) (Lantern.) John L. Fuller and H. Michael Kroll, University of Maine.
33. The effect of smooth muscle stimulants on the vasodilator action of acetylcholine. (15 min.) (Lantern.) Theodore Koppányi, Georgetown University, School of Medicine.
34. Free amino acid in nerve axoplasm. (10 min.) Robert H. Silber and Francis O. Schmitt, Washington University.
35. Role of the auricles in ventricular filling in the tortoise. (6 min.) (Lantern.) Stephen Wood Gray and F. R. Steggerda, Department of Physiology, University of Illinois.
36. Functional results of muscle transplantation in the hind limb of the albino rat. (10 min.) (Lantern.) Roger W. Sperry, The University of Chicago. (Introduced by Paul A. Weiss.)
37. Change of state of the isolated single muscle fiber at a critical length. (15 min.) (Lantern.) Robert W. Ramsey and Sibyl F. Street, Department of Physiology, University of Rochester.
38. The absorption of carbohydrates from the gastro-intestinal tract of brook trout, *Salvelinus fontinalis*. (15 min.) (Lantern.) Arthur M. Phillips, Jr., N. Y. State Conservation Department and C. M. McCay, Cornell University.
39. The secretion of phenol red by the frog renal tubule. (15 min.) (Lantern.) Roy Ph. Forster, Dartmouth College. (Introduced by W. B. Unger.)
40. The effects of increased vascularization and thyroxin on hair growth in under fed albino rats. (10 min.) (Lantern.) Earl O. Butcher, Hamilton College.
41. Effect of extracts of urine from pernicious anemia and gastric cancer patients on gastric secretion. (10 min.) (Lantern.) M. H. F. Friedman, D. J. Sandweiss, R. O. Recknagel and T. L. Patterson, Department of Physiology, Wayne University College of Medicine.
42. Age differences in resistance to CO poisoning among young rodents. (10 min.) (Lantern.) John A. Cameron, University of Missouri.

Section D. *Endocrinology*, 9:30 A.M.; Room 122, Hamilton Hall

43. Further studies on the nature and action of diantlin, a hormone-like substance carried on the spermatozoa of the oyster. (15 min.) (Lantern.) J. B. Allison and T. C. Nelson, Rutgers University.
44. Gonadal stimulation in sexually immature *Fundulus* by implantation of adult hypophyses. (10 mn.) (Lantern.) Samuel A. Matthews, Williams College.
45. The effect of testosterone propionate injections into castrate male frogs, *Rana pipiens*. (15 min.) (Lantern.) Opal M. Wolf, Goucher College.
46. Rudiments of epididymis and vas eferens in female turtles. (10 min.) (Lantern.) Llewellyn T. Evans, University of Missouri.
47. The distribution of intermedin: a new biological method of assay with tests under normal and experimental conditions. (15 min.) (Lantern.) L. H. Kleinholz and H. Rahn, Harvard University.
48. Hormones in the development of the chick. (15 min.) (Lantern.) Nicholas W. Fugo, State University of Iowa. (Introduced by F. A. Stromsten.)
49. The effects of embryonic estrogenic treatment upon sexual characters of adult Brown Leghorn cock. (15 min.) (Lantern.) L. V. Domm, The University of Chicago.
50. Quantitative determination of the follicle stimulating and the luteinizing hormones of the hypophysis. (15 min.) (Lantern.) Emil Witschi, State University of Iowa.
51. On the mechanism and hormones concerned in increase of blood fat in birds. (15 min.) (Lantern.) Oscar Riddle and Tellef Senum.
52. Limitations of food as a factor influencing endocrine reactions in the chick. (15 min.) (Lantern.) W. R. Breneman, Waterman Institute, Indiana University.

THURSDAY AFTERNOON SESSION, DECEMBER 28TH

2:00 P.M., Chapel, University Hall

Joint Symposium with the Genetics Society of America

SPECIATION

Th. Dobzhansky, presiding

- (1) Breeding structure of populations in relation to speciation. Sewall Wright, The University of Chicago.
- (2) Speciation problems in birds. E. M. Mayr, American Museum of Natural History.
- (3) Speciation in *Peromyscus*. L. R. Dice, University of Michigan.
- (4) Levels of divergence in *Drosophila* speciation. Warren P. Spencer, College of Wooster.
- (5) Speciation as a stage in the evolutionary divergence. Th. Dobzhansky, California Institute of Technology.

THURSDAY EVENING SESSION, DECEMBER 28TH

Biologists' Smoker, 9:30 P.M.; Neil House

FRIDAY MORNING SESSION, DECEMBER 29TH

Hamilton Hall, Ohio State University

This session will be held in four sections, as shown below.

Section A. *Symposium, 9:30 A.M.; Room 122, Hamilton Hall*

EXPERIMENTAL STUDY OF CELLULAR ORGANIZATION

M. H. Jacobs, presiding

- (1) Micro-dissection and micro-injection methods. Robert Chambers, New York University.
- (2) Centrifugal force methods. L. V. Heilbrunn, University of Pennsylvania.
- (3) Digestion methods. Daniel Mazia, University of Missouri.
- (4) Permeability methods. M. H. Jacobs, University of Pennsylvania.
- (5) Special optical methods. Francis O. Schmitt, Washington University.

Section B. *General Physiology, 9:30 A.M.; Room 211, Hamilton Hall*

55. Light and reproduction in pheasants. I. Effect of intensity of artificial illumination on the reproductive cycle. ($7\frac{1}{2}$ min.) (Lantern.) Leonard B. Clark, Union College; S. E. Leonard, Rutgers University and Gardiner Bump, New York State Conservation Department.
56. Light and reproduction in pheasants. II. Effect of rate of increase of daily illumination on the reproductive cycle. ($7\frac{1}{2}$ min.) (Lantern.) Leonard B. Clark, Union College and Gardiner Bump, New York State Conservation Department.
57. Reactions of tadpoles to acids and alkalis. (15 min.) (Lantern.) H. T. Folger, Fisk University.
58. The experimental production of melanin pigment on the ventral surface of the summer flounder (*Paralichthys dentatus*). (10 min.) (Lantern.) Clinton M. Osborn, Ohio State University.
59. Activity of melanophores in response to injection of adrenalin. (15 min.) (Lantern.) Madelene E. Pierce, Vassar College.
60. Contrast and melanophore response in fishes. (15 min.) R. N. Danielson, University of Minnesota and Oklahoma Agricultural and Mechanical College. (Introduced by D. E. Minnich.)
61. The active and the resting states of melanophores tested experimentally. (15 min.) (Lantern.) G. H. Parker, Harvard University.
62. The neurohumoral hypothesis and the color changes in the goldfish, *Carassius auratus*. (15 min.) (Lantern.) Sears Crowell, Miami University.

63. Humoral effects of the eyes in amphibian pigment responses. (6 min.) (Lantern.) Stephen W. Gray and Wanda M. Little, Department of Physiology, University of Illinois. (Introduced by F. R. Steggerda.)
64. Color changes in blindfolded American chameleons, *Anolis carolinensis* (Cuvier). (10 min.) (Lantern.) Francis H. Wilson, Tulane University.
65. The mechanism controlling 24-hour cycles in retinal pigment migration in the crayfish. (15 min.) (Lantern.) John H. Welsh, Harvard University.
66. A comparative study of the electrical responses from the compound eye. (15 min.) (Lantern.) Frederick Crescitelli and Theodore L. Jahn, State University of Iowa.
67. A diurnal rhythm in the electrical responses from the compound eye of certain beetles. (15 min.) (Lantern.) Theodore L. Jahn and Frederick Crescitelli, State University of Iowa.
68. Functions of the two types of eye in the brine shrimp, *Artemia gracilis* Verrill. (7 min.) (Lantern.) John H. Lochhead, Harvard University. (Introduced by A. C. Redfield.)
69. Mechanical factors controlling swimming position in aquatic invertebrates and particularly in the brine shrimp, *Artemia gracilis* Verrill. (8 min.) (Lantern.) John H. Lochhead, Harvard University. (Introduced by A. C. Redfield.)

Section C. *Embryology*, 9:30 A.M.; Room 416, Hamilton Hall

70. The respiratory metabolism of normal and genetically deficient eggs of *Drosophila melanogaster*. (10 min.) (Lantern.) E. J. Boell and D. F. Poulson, Yale University.
71. Respiratory metabolism of mammalian eggs and embryos. (10 min.) (Lantern.) E. J. Boell and J. S. Nicholas, Yale University.
72. The oxygen consumption of the chick embryo at various early stages of development. (15 min.) (Lantern.) Fred S. Philips, University of Rochester. (Introduced by B. H. Willier.)
73. Hydrogen-ion concentration of the allantoic and amniotic fluids of the developing chick (15 min.) (Lantern.) Paul A. Walker, University of Connecticut.
74. Size of embryonic organs as detector of organ-specific serological effects. (5 min.) (Lantern.) Paul Weiss, The University of Chicago.
75. Skin transplantation in adult *Rana pipiens*: Autografts compared with homoio grafts. (15 min.) (Lantern.) Howard H. Vogel, Harvard University. (Introduced by Herbert W. Rand.)
76. Observations on species differentials in birds as studied by means of hetero-transplantation of embryonic limb buds. (15 min.) (Lantern.) Herbert L. Eastlick, University of Missouri and The University of Chicago.
77. Alteration and regeneration in the head and pharyngeal regions of planarians. (15 min.) (Lantern.) Frank M. Hull, University of Mississippi.
78. Polydactyl feet in two strains of chicks. (5 min.) (Lantern.) Mary T. Harman and Frances Nelson, Kansas State College.

79. Development of the extra-embryonic circulatory system and the blood picture of chicks incubated under increased atmospheric pressure. (10 min.) (Lantern.) Bert Cunningham and L. J. Flemister, Duke University.
80. Influence of temperature on the rate of early embryonic development. (15 min.) (Lantern.) Alexis L. Romanoff, Department of Poultry Husbandry, Cornell University.
81. Genetic aspects of pigment production in the guinea pig. (10 min.) (Lantern.) Mary T. Harman and Annette Alsop, Kansas State College.
82. Polyembryony and delayed implantation; their occurrence among the genera of armadillos. (10 min.) (Lantern.) G. W. D. Hamlett.

Section D. *Endocrinology*, 9:30 A.M.; Room 312, Hamilton Hall

83. The effect of priming dose of oestradiol and length of the pretrauma period on placentomata size. (10 min.) (Lantern.) Irving Rothchild and Roland K. Meyer, University of Wisconsin.
84. Effects of prospermin on ovaries and uteri of anestrus ground squirrels. (15 min.) (Lantern.) L. J. Wells, University of Missouri.
85. Induction of ovulation in the bat with the pregnancy urine factor. (10 min.) (Lantern.) Mary J. Guthrie and Elizabeth W. Smith, University of Missouri.
86. The experimental shortening of prolonged gestation in the lactating albino rat. (15 min.) (Lantern.) Charles K. Weichert, University of Cincinnati.
87. Effects of adrenalectomy on vaginal and uterine responses to estrogens. (15 min.) (Lantern.) Robert L. Kroc, Indiana University.
88. Function of the luteal-like cells in the mouse ovary. (15 min.) (Lantern.) Carroll A. Pfeiffer and Charles W. Hooker, Yale University.
89. The time and sequence of preovulatory changes in the ovary of the cat following mating or mechanical stimulation of the cervix. (15 min.) (Lantern.) Alden B. Dawson, Biological Laboratories, Harvard University and Harry B. Friedgood, Department of Physiology, Harvard Medical School.
90. The normal reproductive cycle of the female rat as determined by means of the copulatory response. (8 min.) (Lantern.) Richard J. Blandau, John L. Boling and William C. Young, Brown University and Yale University.
91. The time of ovulation in the female rat in relation to the beginning of heat. (7 min.) (Lantern.) John L. Boling, Richard J. Blandau and William C. Young, Brown University and Yale University.
92. Mucus-containing epithelium in the normal guinea pig uterus. (15 min.) (Lantern.) Hugh I. Myers and Helen Rhoda Chornish, University of Richmond.
93. Effects of gonadectomy on the involuted thymus of albino rats. (8 min.) (Lantern.) James C. Plagge, The University of Chicago. (Introduced by Carl R. Moore.)
94. Adrenal, thyroid, and pituitary weights of emotional and non-emotional rats. (10 min.) (Lantern.) Eleanor H. Yeakel and Ruth Pinkey Rhoades, Western Reserve University. (Introduced by Mary E. Collett.)

BUSINESS MEETING

The *Annual Business Meeting of Section F., A. A. A. S.* will be held on Friday, December 29th, 12M.; Room 122, Hamilton Hall. The *Annual Business Meeting of the American Society of Zoologists* will be held in the same room at 12:10 P.M., immediately after the meeting of Section F.

FRIDAY AFTERNOON SESSION, DECEMBER 29TH

DEMONSTRATION PROGRAM

Beginning at 2:00 P.M.; *General Demonstrations*, Rooms 401 and 411, Hamilton Hall. (Motion picture and Kodachrome demonstrations, Room 312, Hamilton Hall.)

Motion picture and Kodachrome demonstrations

151. Functional results of muscle transposition in the hind limb of the albino rat. (8 min.) Roger W. Sperry, The University of Chicago. (Introduced by Paul Weiss.) Motion picture to be shown at 3:00 P.M.; Room 312, Hamilton Hall.
 152. Spiral movement, rhythms and other characteristics of leucoeytic behavior. (6 min.) A. A. Schaeffer, Temple University. Motion picture to be shown at 3:15 P.M.; Room 312, Hamilton Hall.
 153. Kodachrome photomicrographs of color-producing cells of Bermuda coral reef fish. (12 min.) H. B. Goodrich and Marian Hedenburg, Wesleyan University. Kodachrome exhibit to be shown at 3:30 P.M.; Room 312, Hamilton Hall.
- (Demonstrations 151, 152 and 153 will be repeated on request.)

Cytology and Embryology

154. Chromosome divisions in the nurse cells of the ovary of *Drosophila melanogaster*. T. S. Painter and Elizabeth C. Reindorp, University of Texas.
155. Morphological abnormalities of *Drosophila melanogaster* mutants associated with infertility. C. P. Oliver and M. Green, University of Minnesota.
156. Differentiation of chick embryo thyroids in vitro. Esther Carpenter, Smith College.
157. The morphology of limbs developed from homo- and heteroplastic grafts between bird embryos. Herbert L. Eastlick, University of Missouri.
158. The fetal membranes of Insectivora. H. W. Mossman, Department of Anatomy, University of Wisconsin.
159. The fetal membranes of *Dipodomys* (kangaroo rat) and the phylogenetic relationships within the Geomioidea as indicated by these structures. Paul E. Nielson, Department of Anatomy, University of Wisconsin. (Introduced by H. W. Mossman.)
160. Histological and cytological preparations from *Amphiuma*. Francis H. Wilson, Tulane University.
161. Studies on the infiltration of micrological embedding media. Robert T. Hance, Duquesne University.
162. Microscopical characteristics of some embedding media. Robert T. Hance, Duquesne University.

Endocrinology

163. Rest, activity and repair in cortical cells of the pigeon adrenal. Richard A. Miller and Oscar Riddle, Station for Experimental Evolution, Carnegie Institution.
164. Effects of gonadotropic and sex hormones on the urogenital systems of juvenile diamond-back terrapins. Paul L. Risley, State University of Iowa.
165. Correlative cyclical changes in the hypophysis and gonads of *Necturus maculosus* (Rafinesque). I. The male. Henry W. Aplington, Cornell University. (Introduced by Howard B. Adelman.)
166. Effect of sex hormones on the breeding plumage of weaver finches. Emil Witschi and Paul D. Altland, State University of Iowa.
167. Biochemical aspects of the differentiation of the female honey bee (*Apis mellifera* L.). R. M. Melampy, E. R. Willis and R. D. Olsan, U. S. Department of Agriculture and Department of Zoology, Louisiana State University.

General Physiology

168. Apparatus for recording cyclical activity in the rat. John H. Welsh and Leon Levinson, Harvard University.
169. A comparative study of the electrical responses from the compound eye. Frederick Crescitelli and Theodore L. Jahn, State University of Iowa.
170. A diurnal rhythm in the electrical responses from the compound eye of certain beetles. Theodore L. Jahn and Frederick Crescitelli, State University of Iowa.
171. The experimental production of melanin pigment on the ventral surface of the summer flounder (*Paralichthys dentatus*). Clinton M. Osborn, Ohio State University.
172. A study of the duration of the instars of *Daphnia magna* when reared at a constant temperature. J. C. Jenkins and Bertil G. Anderson, Western Reserve University.
173. The substitute for a vascular transport system in *Asterias*. R. A. Budington, Oberlin College.
174. Population fluctuations in *Paramecium* cultures. Edgar P. Jones, University of Akron.
175. Cytolyzing action of *Tarantula* venom. Edgar P. Jones, University of Akron.

General Morphology and Miscellaneous

176. Islet cell types and their topographical relationship to the duct epithelium and exocrine tissue in the pancreas of certain Elasmobranchs. Thurlo B. Thomas, Phillips Exeter Academy.
177. A pronephric kidney combined with a simple mesonephros in a teleost fish, *Macrostoma* (*Lampanyctus*) *warmingi* Lütken. Bradford B. Owen, Harvard University. (Introduced by Alden B. Dawson.)
178. Structure of the skin of the South American frog, *Leptodactylus pentadactylus*. Elbert C. Cole, Williams College.

179. Molting and formation of the exoskeleton in the brine shrimp, *Artemia gracilis* Verrill. John H. Lochhead, Harvard University. (Introduced by A. C. Redfield.)
180. Origin and fate of blood cells in the brine shrimp, *Artemia gracilis* Verrill. Margit Szabo Lochhead, Harvard University. (Introduced by A. C. Redfield.)
181. The venom glands of the black widow spider. Albert M. Reese, West Virginia University.
182. Distribution and color of *Helix hortensis* about Gaspé Bay, Quebec. Stanley C. Ball, Yale University.

FRIDAY EVENING, DECEMBER 29TH, 6:30 P.M.

Zoologists' Dinner, Hall of Mirrors, Deshler-Wallick Hotel

Address by Dr. Wesley R. Coe, vice president of Section F of the A. A. A. S., on
'Divergent Pathways in Functional Development.'

(Open to all zoologists and friends.)

SATURDAY MORNING SESSION, DECEMBER 30TH

Hamilton Hall, Ohio State University

This session will be held in four sections, as shown below.

Section A. *General Physiology*, 9:30 A.M.; Room 122, Hamilton Hall

95. The vacuole system of a fresh water amoeba. (15 min.) (Lantern.) Dwight L. Hopkins, Mundelein College.
96. The measurement of the effect of reduced glutathione upon the initiation of mitosis in *Amoeba proteus* selected in late interkinesis. (15 min.) (Lantern.) H. W. Chalkley, National Cancer Institute, Washington, D. C.
97. The size of the molecules in the growth substance produced by *Chilomonas paramecium*. (15 min.) S. O. Mast and D. M. Pace, Johns Hopkins University.
98. Population fluctuations in *Paramecium* cultures. (10 min.) (Lantern.) Edgar P. Jones, University of Akron.
99. Cytolyzing action of *Tarantula* venom. (5 min.) (Lantern.) Edgar P. Jones, University of Akron.
100. Investigations concerning some of the properties of the natural activator of the enzyme tyrosinase of the grasshopper egg (*Melanoplus differentialis*) with especial reference to the effects of temperature. (7 min.) (Lantern.) J. H. Bodine and L. D. Carlson, State University of Iowa.
101. Cytochrome oxidase during the development of the grasshopper egg (8 min.) (Lantern.) T. H. Allen, State University of Iowa. (Introduced by J. H. Bodine.)

102. The stability of the diapause condition in eggs of the grasshopper, *Melanoplus differentialis*. (7 min.) (Lantern.) W. A. Robbie, State University of Iowa. (Introduced by Titus Carr Evans.)
103. Maximum rates of oxygen transport for certain fresh water fish. (15 min.) (Lantern.) Edgar C. Black, F. E. J. Fry and W. J. Scott, Swarthmore College and University of Toronto.
104. The effect of carbon dioxide on the rate of oxygen uptake by fresh water fishes. (15 min.) (Lantern.) F. E. J. Fry, Edgar C. Black and W. J. Scott, University of Toronto and Swarthmore College.
105. The mechanism of death by cold in the Mycetozoa. (8 min.) (Lantern.) Sister M. Pierre Gehenio and Basile J. Luyet, St. Louis University.
106. Some properties of vitrified muscle fibers. (7 min.) (Lantern; motion picture.) G. Thoenes and B. J. Luyet, St. Louis University.
107. Temperature acclimation and acclimatization in Cladocera. (15 min.) (Lantern.) Katherine K. Sanford and A. M. Banta, Brown University.
108. Panting and temperature regulation in the chicken, *Gallus domesticus*. (10 min.) (Lantern.) W. A. Hiestand and W. C. Randall, Purdue University.
109. Growth rates in the larva of the Mexican bean beetle, *Epilachna varivestris* Muls. (15 min.) (Lantern.) Nellie M. Payne, American Cyanamide Company.
110. Relative growth in the house wren, *Troglodytes domesticus baldwini*. (15 min.) (Lantern.) Sara E. Huggins, Western Reserve University. (Introduced by A. H. Hersh.)

Section B. *Protozoology*, 9.30 A.M.; Room 211, Hamilton Hall

111. A cytological study of *Coelosporidium periplanetae*. (7. min.) (Lantern.) Victor Sprague, University of Illinois. (Introduced by R. R. Kudo.)
112. Observations on the nucleus of *Endamoeba blattae*. (10 min.) (Lantern.) Paul A. Meglitsch, Wright Jr. College, Chicago. (Introduced by Waldo Shumway.)
113. A cytological study of *Blepharisma* with special reference to its fibrillar structures. (8 min.) (Lantern.) C. Leplie Kanatzar, University of Illinois. (Introduced by R. R. Kudo.)
114. Genetic evidence of autogamy in *Paramecium aurelia*. (15 min.) (Lantern.) T. M. Sonneborn, Indiana University.
115. Further observations upon sexual differentiation in *Vorticella microstoma*. (10 min.) (Lantern.) Harold E. Finley, Morehouse College. (Introduced by Lowell E. Noland.)
116. Microscopic anatomy and regenerative behavior of a complex heterotrichous ciliate, *Licnophora macfarlandi*. (15 min.) (Lantern.) William Balamuth, University of California and University of Missouri. (Introduced by W. C. Curtis.)
117. Effects of mechanical agitation on *Paramecium caudatum*. (15 min.) (Lantern.) R. L. King and H. W. Beams, State University of Iowa.

118. The effect of sub-narcotizing and narcotizing concentrations of four primary alcohols on starch production in *Chilomonas paramecium*. (10 min.) (Lantern.) Joseph F. Oliphant, Stanford University. (Introduced by Willis H. Johnson.)
119. The influence of food on the structure of a new species of *Glaucoma*. (15 min.) (Lantern.) George W. Kidder, Brown University.
120. The feeding mechanisms of various ciliated protozoa. (12 min.) (Lantern.) Everett E. Lund, Alfred University.
121. Growth in volume in the ciliate *Ichthyophthirius*. (10 min.) (Lantern.) R. F. MacLennan, State College of Washington.
122. Effects of iodine on growth of the flagellates, *Astasia* and *Euglena*. (15 min.) (Lantern.) J. B. Loefer, H. W. Schoenborn and R. P. Hall, Berea College and New York University.
123. Population studies on pure cultures of a colorless flagellate, *Astasia klebsii*. (8 min.) (Lantern.) Herman Von Dach, Ohio State University. (Introduced by R. C. Osburn.)
124. The mechanism of flagellar and ciliary movement. (12 min.) (Lantern.) Bruce D. Reynolds, University of Virginia.

Section C. *Cytology; Histology; Vertebrate Morphology*, 9:30 A.M.; Room 312, Hamilton Hall

125. Relation between centriole and chromosome numbers in atypical spermatogenesis of *Viviparus malleatus* Reeve. (15 min.) (Lantern.) Arthur W. Pollister and Priscilla Frew Pollister, Columbia University.
126. Chromosome divisions in the nurse cells of the ovary of *Drosophila melanogaster*. (15 min.) (Lantern.) T. S. Painter and Elizabeth C. Reindorp, University of Texas.
127. Effects of 250 r of x-rays on neuroblast mitosis in the embryo of the grasshopper (*Chortophaga viridifasciata*). (7 min.) (Lantern.) J. Gordon Carlson, University of Alabama. (Introduced by C. M. Pomerat.)
128. Cellular movements in the embryo of *Drosophila melanogaster*. (15 min.) (Lantern.) Alfred F. Huettner, Queens College, Flushing.
129. Ultracentrifugation of rat spinal ganglion cells, with special reference to neurofibrillae. (10 min.) (Lantern.) H. W. Beams and H. W. Kirshenblit, State University of Iowa.
130. Cytology of the mammary gland of the bat *Myotis grisescens*. (12 min.) (Lantern.) Katharine R. Jeffers, Duke University.
131. Lymphatic organization in the rabbit appendix. (10 min.) (Lantern.) Edward D. Crabb, University of Colorado.
132. The total distribution of taste buds on the tongue of the pup. (7 min.) (Lantern.) John C. Holliday, Ohio University. (Introduced by Rush Elliott.)
133. Distribution of the taste buds on the tongue of the kitten, with particular reference to those innervated by the chorda tympani fibers of the facial nerve. A preliminary report. (8 min.) (Lantern.) Russell Hayes, Ohio University. (Introduced by Rush Elliott.)

134. The snake eye—an evolutionary 'come-back.' (15 min.) (Lantern.) Gordon L. Walls, Wayne University College of Medicine.
135. Morphological and embryological studies on two species of marine catfish, *Bagre marinus* and *Galeichthys felis*. (15 min.) (Lantern.) Daniel Merriman, Yale University.
136. Studies on starvation in large-mouth bass. (15 min.) Marian F. James, University of Illinois. (Introduced by L. A. Adams.)
137. A basis for a natural classification of fixing fluids. (12 min.) (Lantern.) Wilfrid T. Dempster, University of Michigan.
138. Studies on the infiltration of micrological embedding media. (5 min.) (Lantern.) Robert T. Hance, Duquesne University.
139. Microscopical characteristics of some embedding media. (10 min.) (Lantern.) Robert T. Hance, Duquesne University.

Section D. *Ecology; Parasitology; Miscellaneous*, 9.30 A.M.; Room 416,
Hamilton Hall

140. On a thermodynamic concept of the environment. (10 min.) F. H. Pike, Columbia University.
141. Egg temperatures of wild birds under natural conditions. (15 min.) (Lantern.) Russell A. Huggins, Western Reserve University. (Introduced by J. Paul Visscher.)
142. Deceptive appearance as a factor in tropical ecology. (15 min.) Theodore H. Eaton, Jr., Union College. (Introduced by James W. Mavor.)
143. Distribution of land vertebrates among islands of eastern Lake Michigan. (15 min.) (Lantern.) Robert T. Hatt, Cranbrook Institute of Science.
144. The geographical distribution of digenetic trematodes of fishes of the tropical American Pacific. (10 min.) Harold W. Manter, University of Nebraska.
145. Studies on the morphology and life history of *Spelotrema nicolli* (Trematoda: Microphallidae). (15 min.) (Lantern.) R. M. Cable and A. V. Hunninen, Purdue University, Oklahoma City University and Marine Biological Laboratory.
146. The ecology of the opalinid parasites of Amphibia. (7 min.) (Lantern.) Frank O. Hazard, Ohio State University and Wilmington College. (Introduced by R. C. Osburn.)
147. Studies on the development of the eggs and nauplii of a phyllopod belonging to the genus *Cyzicus* (Estheria). (12 min.) (Lantern.) N. T. Mattox, Miami University.
148. The changes in water content during development of the beetle, *Passalus cornutus* Fabricius. (15 min.) (Lantern.) I. E. Gray, Duke University.
149. The anatomy of *Ferrissia tarda*. (10 min.) (Lantern.) C. Clayton Hoff, University of Illinois. (Introduced by H. J. Van Cleave.)
150. A pre Linnean system of binominal nomenclature as developed by the Tupi Indians. (5 min.) (Lantern.) G. W. D. Hamlett.

PAPERS PRESENTED BY TITLE

Embryology

183. On the effects of extreme cold and high vacuum on the development of *Artemia* cysts. D. M. Whitaker, Stanford University.
184. Mosaic development in the Annelid egg. D. P. Costello and H. M. Costello, University of North Carolina.
185. Studies of fertilization. I. The fertilizability of nucleated and non-nucleated fragments of centrifuged *Nereis* eggs. D. P. Costello, University of North Carolina.
186. The structure of the polar body and egg cortex in *Chaetopterus*. Leonard G. Worley, Brooklyn College.
187. The behavior of the dictyosomes during cleavage and gastrulation in *Arbacia*. Leonard G. Worley, Brooklyn College.
188. Microdissection of a ciliated epithelial cell. Leonard G. Worley, Brooklyn College.
189. Abnormalities produced in *Tribolium confusum* Duval by exposure to a secretion of their own manufacture. Louis Roth and Ruth B. Howland, Washington Square College, New York University.
190. Effects of early centrifugation on *Drosophila* eggs, embryos, larvae, pupae and adults. Ruth B. Howland, Harry G. Albaum and Samuel Kaiser, Washington Square College, New York University and Brooklyn College.
191. The temperature-effective-period at 16°C. for the genotype vg^{N03}/vg^p of *D. melanogaster*. Adele I. Cohen and Morris H. Harnly, Washington Square College, New York University.
192. The effect of distilled water and iced distilled water on the viability of developmental stages of *Rana pipiens*. Linden F. Edwards, Ohio State University. (Introduced by Clinton M. Osborn.)
193. Abnormalities in frog embryos induced by centrifugation. T. W. Torrey and W. R. Breneman, Indiana University.
194. Androgenetic hybridization using *Rana brachycephala* (Cope) and *Rana pipiens* Schreber. K. R. Porter, Princeton University.
195. Hybridization in frogs. John A. Moore, Columbia University. (Introduced by A. W. Pollister.)
196. Glycogenetic activity in early stages of the developing amphibian. Martha Revel Barnes, University of Illinois. (Introduced by Waldo Shumway.)
197. Developmental rate and alternating temperature. J. William Buchanan, Northwestern University.
198. The oxygen consumption of the amphibian organizer. L. G. Barth, Columbia University.
199. Neural differentiation without organizer. L. G. Barth, Columbia University.
200. Experimental analysis of the development of the anuran olfactory organ. Edgar Zwilling, Columbia University. (Introduced by L. G. Barth.)
201. Experiments on the 'determination' of the fin ectoderm in salamander embryos. Victor C. Twitty, Stanford University.
202. Embryonic induction in regenerating tissue of *Rana pipiens* and *Rana clamitans* larvae. Henry S. Emerson, Yale University and University of Michigan. (Introduced by W. T. Dempster.)

203. Forelimb opercular perforation in *R. catesbeiana*. O. M. Helff, New York University.
204. Studies on the anuran respiratory system. I. Histological changes in gill and lung tissue during growth and metamorphosis. C. M. Harrold, Jr., New York University. (Introduced by O. M. Helff.)
205. Further studies on regeneration in the tail fins of *Fundulus heteroclitus* embryos. S. Milton Nabrit, Atlanta University.
206. Further studies on regeneration in the central nervous system of *Fundulus heteroclitus* embryos. S. Milton Nabrit, Atlanta University.
207. Surface tension of the allantoic and amniotic fluids of the developing chick. Paul A. Walker, University of Connecticut.
208. Reciprocal heteroplastic chorio-allantoic transplantations of macerated chick and duck kidney tissue. Carl J. Sandstrom, Herman N. Eisen, and Robert S. Siffert, University College, New York University.
209. Retrogression of the avian mesonephros in chorio-allantoic grafts. Carl J. Sandstrom, University College, New York University.
210. The effect of testosterone propionate on the early development of the reproductive ducts in the female sparrow hawk (*Falco sp. sparverius*). Olin E. Nelson and Robert M. Stabler, University of Pennsylvania.
211. The embryonic and post-natal development of the female prostate gland in the albino rat. John J. Mahoney, State University of Iowa. (Introduced by Emil Witschi.)
212. Reproductive phenomena in the mink (*Mustela vison*). Robert K. Enders, Swarthmore College.
213. The influence of nerve fibers upon taste buds during embryonic development. Theodore W. Torrey, Indiana University.

Endocrinology

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289. The collateral circulation in the thigh of the cat. Homer B. Latimer and Tom G. Orr, Jr., University of Kansas.
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292. Spontaneous outgrowths in *Dugesia tigrina* (syn. *Planaria maculata*). E. D. Goldsmith, Washington Square College, New York University.
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ABSTRACTS

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PAPERS READ

1. THE EFFECT OF SOME INHIBITORS OF CARBOHYDRATE METABOLISM ON THE DEVELOPMENT OF THE FROG EGG. C. M. Pomerat and R. Haringa, Clark University and the University of Alabama. (Lantern; 8 min.)

Eggs of *Rana pipiens*, at various stages of development, which were obtained by pituitary stimulation, were placed in solutions known to inhibit carbohydrate metabolism at specific concentrations.

Sodium fluoride in distilled water inhibited the development of eggs freed of jelly at M/50 but cleavage proceeded normally at M/200. At this concentration muscular movement of young tadpoles seemed retarded. Cleavage was inhibited by iodoacetic acid at approximately M/1500 while mono-iodo-acetamide at M/20,000 arrested cleavage without cytolysis in stages up to and including the closure of the blastopore. Beginning with the neurula inhibition did not take place as rapidly, but embryos cytolysed. The neural canal was never observed to close in the presence of M/20,000 acetamide. No difference was observed between eggs in 0.005 M glyceraldehyde and control eggs in pond water. This is probably due to the rapid destruction of glyceraldehyde by the living egg.

These results are consistent with the view advanced by Needham and his associates that the energy for early development is provided by a very active non-phosphorylating glucolytic system.

2. THE CAPSULAR FLUID OF AMBLYSTOMA PUNCTATUM EGGS. Oscar W. Richards. Research Department, Spencer Lens Co. (Lantern; 10 min.)

The capsular fluid was carefully collected from eggs at Harrison stages 18-22 and 28-32 and analyzed for Na, Ca, K, Cl, and total protein content. The following physical properties were also determined: specific gravity, surface tension, osmotic pressure, bound water and viscosity. The protein content is mucin which does not support bacterial growth; although occasionally an alga may grow within the capsule. Comparison with Ringer and Holtfreter solutions shows that these substitutes for the capsular fluid are hypertonic. They also lack the protein content of the natural fluid. The fluid at these two stages will be compared. From this data solutions may be made which more nearly resemble the natural capsular fluid for the development of eggs following the operative procedures of the experimental embryologists.

3. THE POTENCY OF THE ISOLATED VEGETAL HEMISPHERE (PRESUMPTIVE ENDODERM) OF THE BLASTULA OF AMBLYSTOMA PUNCTATUM. Louis T. Stableford, Yale University. (Introduced by J. S. Nicholas.) (Lantern; 12 min.)

The vegetal hemisphere of the blastula, isolated by aspirating the micromeres and marginal zone through a slit in the vitelline membrane, was suspended, within

the membrane, in sterile agar-Holtfreter's solution. The tissue became a hemisphere, the round blastocoel-floor cells formed a pigmented pavement, and in the decided majority of cases a projection of small cells appeared. Two cases showed pulsating structures.

A study of sections shows: (1) two types of endoderm—typical yolk mass with cells, and foregut endoderm; (2) ectoderm, with foregut endoderm, forming a covering for the experimental mass; (3) mesoderm which is most prominent in the projection region but which extends along the bottom of the mass—in some cases through its entire length. It is also found in isolated aggregates in the yolk mass. Older preparations show muscle cells, blood vessels, blood islands, pronephros-like structures, and chorda; in all cases somite formation is found. The degree of histogenesis closely parallels that of the controls.

A tissue which normally possesses limited potency has produced the three primary tissues. Special precautions were taken to avoid the possibility that this tri-potency is due to remnants of the marginal zone remaining in the preparations. There are either presumptive areas deep in the blastula which cannot be charted by vital staining methods or the presumptive endoderm has a threefold potency.

4. THE ALTERATION OF DEVELOPMENTAL PATTERN IN SAND DOLLAR EGGS WITH PILOCARPINE. Olin Rulon, Wayne University. (Lantern; 15 min.)

By controlling the concentration ($m/100\text{-}m/5000$), the length of the exposure period, and the period in the developmental history when the eggs are exposed, the developmental pattern of the sand dollar (*Dendraster excentricus*) can be altered in many different ways with pilocarpine.

With proper treatment dorso-ventrality will not appear and the developing larvae remain radial although differentiating in many respects. Skeleton can be caused to form in over-abundance or not at all. It is possible to produce exogastrulae with retarded or increased endoderm. The oral lobe can be grossly inhibited or caused to be vastly larger than normal. With certain treatment anal arms will develop at widened angles to each other, asymmetrical, askew, or not at all. Under proper conditions a large ciliated knob can be made to appear dorsal to the region of an inhibited oral lobe or at the extreme apical end of an exogastrula.

These and other modifications all point to a dynamic polar gradient as the primary pattern of normal development with different susceptible regions of partial physiological isolation appearing as development proceeds. All of the modifications noted seem to result from differential inhibition, recovery, conditioning, or combinations of these factors depending upon the conditions of the experiment.

5. A COMPARISON OF THE DEVELOPMENT OF ARBACIA HALF-EGGS ACTIVATED BEFORE AND AFTER CENTRIFUGATION. Ethel Browne Harvey, Princeton University. (Lantern; 15 min.)

After Arbacia eggs have been fertilized and the fertilization membranes removed, they may be broken into two fairly uniform unequal halves by centrifugal force. The upper white halves (containing both ♂ and ♀ nuclei) sometimes cleave normally and develop into blastulae but rarely into plutei and these are abnormal. The lower red halves (containing no nuclei) do not cleave at all and there is no development, although there is some evidence of activation. These non-nucleate

halves from fertilized eggs cannot be again activated either by sperm or artificially, by salts. It seems to make little difference at what stage after fertilization the eggs are broken apart, whether before or after the breakdown of the nuclear membrane.

On the other hand, similar half eggs (with similar nuclear content) obtained by centrifuging the unfertilized egg and subsequently fertilizing or activating them, develop much better. The upper white halves when fertilized (containing both the ♂ and ♀ nuclei) usually cleave quite normally and develop into normal white plutei of half normal size. The lower red halves when artificially activated (containing no nuclei, 'parthenogenetic merogones') may cleave fairly regularly and develop as far as blastulae which emerge from the fertilization membranes.

6. EVIDENCE OF ROLE OF SULPHYDRYL GROUP IN PHENOMENA OF FERTILIZATION. Grace Townsend, Marine Biological Laboratory. (Introduced by Ralph Lillie.) (Lantern; 10 min.)

Previous work has linked the spawning inducing material from ripe *Nereis* limbata eggs, postulated as essential to fertilization (Lillie '13, Townsend '39) with glutathione (Townsend '38). The present work suggests the possible role of sulphhydryl compounds in: (a) sperm activation, (b) sperm penetration, (c) membrane elevation, and (d) aster formation initiating cleavage.

Glutathione (1:3000 parts sea water) activates sperm, higher concentrations induce clumping.

Glutathione (1:500) melts the cortical gel of *Arbacia* eggs and renders their surfaces 'sticky', foreign cells adhere and appear to penetrate the surface. Fertilization following brief treatment produces very exaggerated fertilization cones. Sperm have a high sulphhydryl content (400 mg. per 100 gm.), the reducing properties may induce the fertilization cone.

Glutathione (1:1000-1:2000) induces partial parthenogenesis of *Nereis* eggs; lesser concentrations prolong fertilizability, also restore fertilizability to washed eggs. Pre-fertilization treatment of *Arbacia* eggs produces wider and thicker membranes. Glutathione removes the copper block to membrane elevation.

Lillie associated the rupture of the germinal vesicle with the freeing of a fertilization essential substance, Mathews ('07) with aster formation. Chalkley ('37) found nuclear rupture in ameba to free a nitro-prusside positive substance. The germinal vesicle of *Nereis* eggs gives a strongly positive nitro-prusside test. Glutathione, under conditions favorable to oxidation, can gel cytoplasm, a process involved in aster formation.

7. THE EFFECT OF GLUTATHIONE ON SENSITIVITY OF ARBACIA GERM CELLS TO X-RAY CONSIDERED FROM THE VIEWPOINT OF CHILD'S GRADIENT THEORY. Grace Townsend, Marine Biological Laboratory. (Introduced by Ralph Lillie.) (Lantern; 5 min.)

In studying the effect of variable concentrations of glutathione on various marine ova and sperm it was noted that the effects of high concentrations was the same as produced by x-ray and ultra-violet rays on the respective materials (Redfield '21, Henshaw '32, '33, '38, '39) and by photosensitive dyes (Hinrichs '26). It was therefore of interest to test if gametes may become more sensitive

to x-ray when immersed in variable concentrations of glutathione. Arbacia eggs and sperm were selected as well standardized material.

An increase in the sensitivity of the ova and sperm to x-ray in the presence of glutathione was indicated by: (a) additional lengthening of the period of early prophase with correspondingly greater delay in first cleavage, (b) an increase in the percentage of eggs showing multipolar cleavage and cortical deforming ruptures, (c) an increase in the percentage and degree of deformed larvae, (d) an increase in the percentage of eggs failing to cleave and an increased death rate of larvae. The delay in cleavage was greater than the sum of the delays induced by the separate agents. Eggs in glutathione solutions following irradiation showed less recovery than similarly treated eggs in sea water.

Child and Deviney ('24) and Hinrichs ('26) have found evidence that the susceptibility of organisms to deleterious radiation is related to the metabolic 'gradient pattern', the active regions being most susceptible to inhibitive dosage. Glutathione is a typical constituent of active regions.

Glutathione treatment of eggs increases their viscosity, and may be presumed to affect their reduction potential.

(I wish to thank Dr. Paul S. Henshaw for counsel and assistance in this work.)

8. THE EFFECT OF COLCHICINE ON CYTOPLASM AND NUCLEI OF THE ZEBRA FISH BLASTOMERES. Edward C. Roosen-Runge, Brown University. (Lantern; 15 min.)

The living eggs of the zebra fish were observed individually after treatment with colchicine in the early cleavage stages. The results were confirmed by the observation of sectioned material.

It was found that the first effect of colchicine was on the cytoplasm, particularly on the surface of the cells. Colchicine in a concentration of 1:3000 damages the formation of furrows in 90% of the cases. The furrows may be entirely suppressed. The asters and the spindles are injured by stronger concentrations or by prolonged treatment. The nuclei are usually the last to be affected. Different abnormalities of the nuclei were found, consisting either of condensation and disintegration or of the formation of giant nuclei. However, in many cases the abnormal nuclei were observed to undergo periodic changes, which corresponded in time exactly to the periodic changes observed in the normal mitotic cycle.

9. EXPERIMENTS ON POLARITY DETERMINATION IN TUBULARIA REGENERATES. James A. Miller, University of Michigan. (Lantern; 15 min.)

A differential with respect to opportunity for metabolic exchange has been suggested as determining polarity in a large number of cases. However, there is little direct evidence that a local increase of oxygen will actually cause development of apical structures, or metabolites prevent it. Such data have been obtained by allowing *Tubularia* stems to regenerate in partitioned chambers with their ends exposed to different concentrations or agents.

With long pieces reversal of polarity occurred in all stems with their proximal ends on the oxygenated side, when the other side of the partition was treated with boiled water, nitrogen, CO₂, or with a 90% O₂ + 10% CO₂ mixture. Short pieces gave similar though less striking results. With the same oxygen tension on both sides, circulation alone was sufficient to modify polarity, as also were temperature

differentials. The above suggests that any modification of the general metabolism may be expected to modify or even determine hydranth reconstitution at an exposed surface.

Oxygenation of 1 mm. pieces gave marked increases of bipolars, reaching 100% in some cases. Concomitantly there was an increase in percentages of partial hydranths over wholes, both unipolar and bipolar. It is significant that every partial hydranth examined had distal tentacles and that they were largest in the most incomplete regenerates. It follows that the most distal part of the reconstituting hydranth is primary and that the presence and size of proximal structures depends on the scale of organization of the distal and on available materials.

10. SOME PROCESSES OF DEVELOPMENT IN THE POSTERIOR END OF THE LAMPREY EMBRYO ANALYSED BY LOCALIZED VITAL STAINING. Richard Weissenberg, The Wistar Institute. (Introduced by Clyde E. Keeler.) (Lantern; 15 min.)

As shown in earlier investigations, the gastrulation of the lamprey (*Lampetra fluviatilis*), represents a genuine invagination (Weissenberg 1933, 1934, 1936). The marking of areas of the posterior end of the early neurula has now given the result that the invagination continues during the neurula stage until the first appearance of the head process. The circumference of the blastopore contracts eccentrically ventrad and forms the anus. The last part of the archenteron becomes the anal gut. The tail-mesoderm is connected with the sidewalls of the anal gut and forms dorsolateral wings.

The prospective tail-mesoderm is situated in the equatorial zone of the blastula as a right and a left arch which extend from dorsolateral to ventrolateral representing parts of the anal gut-anlage. The fact that the ventrocaudal area of the equatorial zone of the blastula contributes to the formation of the tail-mesoderm shows that the diagrams outlined hitherto for the localization of the prospective organs of the lamprey were incomplete regarding the range of the mesoderm zone. In this respect a diagram of the anlage zones for the lamprey blastula would now have more resemblance to the diagram outlined by W. Vogt for *Anura*. But there remain as essential differences that in the lamprey no median ventral mesoderm exists and further that the tail-mesoderm-anlage is here not restricted to the ventrolateral zone. The whole mesoderm of the lamprey represents in the opinion of the author an archenteron-component with primary connection with the gut-entoderm.

11. DEVELOPMENT OF THE NETWORK OF THE ADEPIDERMAL MELANOPHORES IN THE TADPOLES OF *DISCOGLOSSUS PICTUS*. Hans Elias. (Introduced by Frederic T. Lewis.) (Epidiascope; 12 min.)

Urodela, both larvae and adults, have adepidermal melanophores—large, flat, movable cells. Adepidermal melanophores are transformed, in the primitive anuran family *Discoglossidae*, into big, cylindrical or thread-shaped, rigid cells, which form polygonal or rectangular networks, with a supporting function (elasticity modulus 190 kg/cm²). Being no longer movable and flat, they are replaced in their color-regulating function by subcutaneous melanophores in larvae, and by intracutaneous melanophores in adults; and subsequently, in higher forms of *Discoglossidae*, they lose their pigment. Having lost their original function and

being superfluous in their new one, they disappear in toads and are not present at all in frogs.

Adepidermal melanophores form in *Discoglossus* a continuous, polygonal network. They arise from cells of the corium adherent to the basement membrane of the epidermis. At first they are separate from one another, but later join in the network.

Active growth in length of their spurs occurs only as long as they terminate freely. As soon as they reach another adepidermal melanophore, they adhere to it, and further increase in length occurs only passively, by the traction exercised by one melanophore on another, due to the growth of the epidermis.

Thus there is shown a purely mechanical regulation of this melanophoric tissue, in its dimensions and structure, by the growth of another organ, the epidermis. An organ which has the power thus to regulate the development of another may perhaps be called a regulator.

12. THE EFFECT OF CALCIUM ON THE PHYSICAL PROPERTIES OF THE ENDOTHELIAL CEMENT. Robert Chambers and B. W. Zweifach, Laboratory of Cellular Physiology, New York University. (Lantern; 5 min.)

In a study of the blood capillaries of the frog's mesentery the following data was obtained regarding the nature of the material between the endothelial cells:

(1) A 10% solution of AgNO_3 sprayed on the capillary with a micropipette resulted in the immediate blackening of the endothelial lines or intercellular cement of the endothelium while the blood flow continued undisturbed. (2) A suspension of carbon introduced into the blood stream by way of the heart results in the adhesion of carbon exclusively to the intercellular cement. This indicates that cement is normally sticky while the surface of the endothelial cells is not. (3) Perfusion of the circulation with calcium-free Ringer containing 0.5 to 0.7% ash-free gelatine causes the adhering carbon to be washed away and a marked loss of fluid to occur. This is enhanced by acidifying the perfusion fluid to about a pH 6.4. The leakiness of the capillaries is strikingly demonstrated by suspending washed rooster red cells in the perfusion fluid, whereupon rapid and extensive extravasation of the cells occurs. (4) Reconstitution of normal conditions in the capillaries occurs by replacing the calcium-free perfusate with one containing calcium. Doubling the amount of calcium normally present in Ringer causes a marked overall stickiness of the inner surface of the capillary. (5) The dispersion of the intercellular cement is accelerated by decreasing the pH.

Conclusion. The intercellular cement is continually being excreted by the endothelial cells. With AgNO_3 it forms an irreversible salt. With calcium it forms an reversible salt having strongly cohesive and sticky properties. In the absence of calcium it forms a soluble salt which readily washes away.

13. ALTERATIONS IN THE PHYSICAL PROPERTIES OF THE RED CELL. Benjamin W. Zweifach, Laboratory of Cellular Physiology, New York University. (Introduced by M. J. Kopac.) (Lantern; 10 min.)

The red blood cell of the frog is quite fragile in the blood stream so that when prodded with the point of a fine micro-needle, the cell, except for the nucleus, suddenly disappears. The cell can be compressed with a blunt needle but cannot

be stretched to any extent without injury. When erythrocytes are suspended outside the body either in saline or Ringers, no sudden disruption follows prodding of the cell. The erythrocyte is less flexible and a gradual hemolysis occurs leaving a ghost behind.

Stretching, repeated buffeting, long exposure to tissue fluids or to venous blood results in a gradual change in the plasticity of the red cell and in its reaction to prodding. The cell tends to be converted into a more rigid body with an increasingly visible nucleus but with no apparent hemolysis. At this stage, the altered cell can be stretched between two needles. This is not a property of the normal red cell, the tensile strength being due to the persistence of an elastic coagulum. In cases where the red cell is caught in the endothelial wall, the portion of the erythrocyte remaining in the stream is pulled out by the force of the blood flow and often irreversibly stretched into a long filamentous stalk with a hemoglobin-containing bleb on its free end. A similar type of permanent deformation occurs when a red cell is trapped in the lumen of a constricted capillary for periods of at least 3 to 5 minutes.

14. CHEMICAL AND STRUCTURAL FEATURES OF THE SURFACE OF RED CELLS AND GHOSTS. A. K. Parpart and A. J. Dziemian. Princeton University. (Lantern; 5 min.)

Further studies on the chemical composition of the surface of red cells have proceeded along two lines and have indicated possible structural features of the surface. 1. The ratio of lipid to non-lipid (protein) compounds in the cell surface is of the order of 1 to 2. This has been determined on the ghosts of three different species of mammals. These data are not in accord with recent reports in the literature. 2. The possibility of lipo-protein complexes in the surface of the red cell has been investigated. Such complexes appear to be present to variable extent dependent upon the species used. The amount of lipo-protein in the cell surface of the species examined range in the order, beef > rabbit > rat. These lipo-proteins in the surface structure might be expected to alter the number and distribution of aqueous channels in the cell surface and thus decrease the permeability to non-lipid soluble compounds. As has frequently been shown the permeability of the red cells of these mammals range beef < rabbit < rat.

Some preliminary results confirm the suspicion that cephalin and lecithin moieties are primarily concerned in the lipo-protein complex formation. These results strongly suggest that the architecture of the cell surface as well as its chemical composition, plays an important part in its function.

15. THE ACTION OF LIPASE ON THE CELL SURFACE. R. Ballentine and A. K. Parpart, Princeton University. (Lantern; 10 min.)

The action of pancreatic lipase on the permeability of red cells was examined in an attempt to make a further correlation between the composition of the cell surface and its permeability. Cells were exposed to highly active pancreatic lipase at optimum pH for several hours before examining their permeability to a variety of compounds. The permeability of beef erythrocytes was increased to substances which penetrate both primarily as molecules and primarily as ions. The lytic

action of hemolytic concentrations of alcohols was also increased. Compounds which are thought to penetrate by virtue of their lipid solubility, the fatty acids, did not show any change in their rate of entrance as compared with control cells.

These results would indicate that the lipase has removed some lipid from the cell surface, possibly a portion of the lipid in the lipo-protein complex, and thus created more aqueous channels for the penetration of non-lipid soluble compounds. At the same time the action of hemolytic alcohols would be facilitated.

16. THE EFFECT OF TEMPERATURE ON CELL PERMEABILITY AND ON CELL RESPIRATION. F. R. Hunter, Rhode Island State College. (Lantern; 10 min.)

Chicken erythrocytes exposed to a temperature 15-18°C. above normal for one half to three-quarters of an hour, respire at a much lower rate when returned to 37°C. These cells are more permeable to substances such as glycerol. The heat treatment causes an increase in the size of the cell and an increase in their fragility.

17. QUANTUM REQUIREMENTS FOR PHOTODYNAMIC HEMOLYSIS. Harold F. Blum, National Cancer Institute, United States Public Health Service, and Howard W. Gilbert, Washington Biophysical Institute. (Introduced by C. B. Davenport.) (Lantern; 15 min.)

Measurements have been made of the amount of monochromatic light energy required for photosensitized hemolysis when the time occupied by the hemolytic process is excluded. These measurements show (1) strict adherence to the reciprocity law, showing that the measurements are an index of the elementary photochemical mechanism; (2) the number of absorbed quanta required to hemolyze a red blood cell is the same regardless of the concentration of the dye on the cell membrane, demonstrating that hemolysis occurs when a minimum amount of damage has been done to the cell membrane; (3) the number of quanta absorbed per dye molecule varies from a few to thousands. Hence the dye must act in a cyclic process in which it serves to transfer the absorbed energy to a chemical reaction without being itself altered at the end of the reaction. This agrees with the concept that the mechanism leading to hemolysis is a photosensitized oxidation of the type which is common in more simple systems. The accumulated evidence cannot be harmonized with any other mechanism.

18. SPIRAL MOVEMENT, RHYTHMS AND OTHER CHARACTERISTICS OF LEUCOCYTIC BEHAVIOR. A. A. Schaeffer, Temple University. (Lantern; Motion picture; 15 min.)

Polymorphonuclear leucocytes of the frog, chicken, mouse, rat and man move spirally in capillary glass tubes. The general characteristics of spiraling are very similar to those of amebas. In man, the ratio of left turns over right turns varies from day to day in a rhythmic and predictable manner. Rhythms are fairly regular in any one individual. The length of rhythms in different individuals varies from 11 to 14 days. In a normal individual 80 to 90% of the leucocytes in a blood sample show approximately the same steric ratio. Focal infections and

apparently the anomalistic (rather than the tropical) lunar month affect the rhythms, presumably through the introduction of new leucocytes into the blood stream or through some change in their metabolism. Insolation increases the rate of movement, the amount of spiraling in unit time, the average length of sections of path consisting of one direction only, and in general, it results in more highly coordinated movement.

Re-examination of old data on spiraling amebas suggests strongly the presence of similar rhythms in these organisms.

19. THE STIMULATING ACTION OF CITRATES AND OXALATES ON THE NEREIS EGG.

Karl M. Wilbur, University of Pennsylvania. (Introduced by L. V. Heilbrunn.) (Lantern; 15 min.)

Previous work has shown that various chemical and physical agents stimulate the egg of *Nereis limbata* to undergo a series of reactions, one of which is the breakdown of the germinal vesicle. The process of germinal vesicle breakdown has proved a convenient reaction for the study of stimulation and response in a relatively simple protoplasmic system. This response, which normally follows stimulation, can be reversibly inhibited by first treating the eggs for a few minutes with 0.35 M sodium citrate. In spite of the fact that citrate solutions exert an inhibitory action, under some conditions citrate may also act as a stimulating agent. This can be demonstrated by immersing eggs in solutions of sodium citrate, potassium citrate or potassium oxalate mixed with sea water. In certain mixtures the eggs undergo germinal vesicle breakdown, polar body formation and irregular cleavage. Ammonium citrate and oxalate solutions mixed with sea water give very little germinal vesicle breakdown and usually produce cytolysis.

The action of mixtures of sodium or potassium citrate and sea water, like that of other stimulating agents, is prevented if the eggs are first treated with an isotonic solution of sodium or potassium citrate.

20. VISCOSITY AND GELATION IN THE ARBACIA EGG. Robert Chambers, Laboratory of Cellular Physiology, New York University. (Lantern; 6 min.)

It has long been known from Heilbrunn's centrifuging experiments that the viscosity of the cytoplasm progressively increases from the time of insemination until a peak is reached which tends to coincide with the period of the fully developed sperm-aster.

It has also long been determined that a progressively increasing region of gelation occurs as expressed by the growing sperm-aster until, when the aster is fully developed the gelation involves most of the substance of the egg-cytoplasm.

In recent experiments it has been ascertained that the increase in viscosity is independent of the gelation which produces the aster. Centrifuging tends to destroy the aster without affecting the viscosity. Moreover, an increase in viscosity is detectable while the aster is still a diminutive body and the viscosity remains high during the streak stage when the aster has faded out. Again, the viscosity is low during the amphiaser stage and does not rise until after the cleavage has been completed.

Evidently, therefore, the increased stiffness occasioned by gelation (aster) is not necessarily related to the changes in viscosity of the cytoplasm, the viscosity not being affected by centrifugation.

21. THE SUSPENSION OF ACTIVITY IN THE FERTILIZED ARBACIA EGG BY EARLY IMMERSION IN KCl. E. L. Chambers and Robert Chambers, Laboratory of Cellular Physiology, New York University. (Lantern; 4 min.)

In a recent report (Biol. Bull., 1938, 75, 356) on the action of KCl on inseminated Arbacia eggs three periods of reactivity to the salt were noted, when the eggs were immersed in the salt solution at intervals of time between insemination and first cleavage: an early and a late reactive period and a middle non-reactive period.

The present paper deals with the first reactive period which lasts for several minutes after insemination. The effect is an immediate cessation of all visible activity. If the inseminating spermatozoan has not yet entered, it remains attached to the surface of the egg and the elevation of the fertilization membrane is not uncompleted.

If the sperm has already entered, the sperm nucleus makes no further advance and the peripheral migration of the pigment granules does not take place. The eggs can be left in this suspended condition for over 45 minutes in the KCl solution with a resumption of activity and normal development when returned to sea water.

22. FACTORS AFFECTING RATE OF CLEAVAGE IN ARBACIA; A PRELIMINARY STUDY OF HOMOTYPIC EXTRACTS. W. C. Allee and Asher J. Finkel. The University of Chicago. (Lantern; 15 min.)

Allee and Evans (1937) demonstrated that, with various experimental conditions, eggs of *Arbacia punctulata* cleave more rapidly in relatively dense than in relatively sparse populations. In an attempt to discover the possible factors involved, extracts of *Arbacia* eggs were prepared. Of these an alcohol extract, made by treating *Arbacia* eggs (in the 8-cell stage) with 95% ethanol, evaporating the ethanol fraction, and taking up the residue in sea water, appeared to be the most potent in accelerating early cleavages. This extract, added to sea water in dilutions ranging from 1 part in 2×10^7 to 1 part in 2×10^{12} , seemed to be most effective in concentrations of $\frac{1}{2 \times 10^7}$ to $\frac{1}{2 \times 10^8}$, although acceleration

of cleavage was noted even in the greater dilutions, notably $\frac{1}{2 \times 10^{11}}$. In the former set of concentrations, 99 paired comparisons, between populations of eggs placed in 20 mm.³ of sea water and killed serially during the progress of second cleavage, showed that the eggs treated with the extract were ahead of their controls. The differences between the percentages which achieved second cleavage at the time of killing averaged 10.59 with $P < 0.0000001$; this is highly significant statistically. Twenty-one paired comparisons, selected only for being near mid-second-cleavage for the same concentrations, showed a similar positive difference for the extract of 10.36 with a significant P value of 0.0025. It seems therefore that substances, easily prepared from *Arbacia* eggs, and when present in sea water in extremely dilute concentrations, can accelerate the early development of *Arbacia* eggs.

23. THE EFFECT OF MONOCHROMATIC ULTRA-VIOLET RADIATION ON THE EGGS OF CERTAIN INVERTEBRATES. Alexander Hollaender, Myrna F. Jones, and Leon Jacobs. Divisions of Industrial Hygiene and Zoology, National Institute of Health. (Introduced by Mary Juhn.) (Lantern; 10 min.)

When eggs of invertebrates are exposed to measured quantities of monochromatic ultra-violet radiation in such a manner that each egg receives an equivalent amount of energy over the entire surface of the egg, a variety of effects can be observed. The lethal effect is the most obvious one to be obtained. This can be caused by destruction of the entire cell or embryo, or by interference with the functions and structures necessary for development.

A careful study of the effects of sublethal dosages invariably shows a slowing down of the development of the cell or embryo, a modification of the morphological structures, and certain delayed effects. Radiation can also produce parthenogenesis in a similar manner to chemical and physical agents. These lethal and sublethal effects will be discussed on the basis of work done by the authors on the eggs of *Enterobius vermicularis* (Nematoda) and *Arbacia punctulata* (Echinoidea), and also on the basis of work on related organisms by other investigators (Heilbrunn, Bodine, Giese, and others). Wavelength dependence curves for the effects of ultra-violet radiation will be shown.

24. EFFECTS OF TEMPERATURE ON THE RADIOSENSITIVITY OF CELLS. T. C. Evans, State University of Iowa. (Lantern; 8 min.)

This report deals with the effects of roentgen radiation in delaying cleavage and preventing normal development of eggs of *Ascaris equorum*. It was found that at low temperatures the cleavage delay resulting from irradiation was proportional to the dosage within the limits employed (1000 to 16,000 r). At the higher temperatures the delay was not proportionately increased by heavier irradiation. Exposure to cold for several weeks following irradiation resulted in the delay being more proportional even at the higher dosages.

Exposure to low temperature during and following the irradiation increased the number of eggs able to form normal embryos.

25. X-RAY EVIDENCE FOR THE REALITY OF VIRUS PARTICLES OF LARGE SIZE. John W. Gowen, Iowa State College. (Lantern; 15 min.)

Four wave lengths of x-rays have been used on certain viruses, bacteria and biologically more complex cellular organisms to study the question—is size of organism directly related to the amount of the observed inactivation. The average wave lengths of these different treatments determined by absorption experiment in al. were 2.1 Å, 1.5 Å, 0.7 Å and 0.03 Å. The results show the 0.7 Å wave length most effective, the 1.5 Å next, and the 2.1 and 0.03 Å least. This result suggests a maxima between these limits.

The form of the inactivation curve of the viruses is the same as that for many other biological materials, one absorption of energy being sufficient to cause the inactivation of the virus particle. These changes are unaffected by the presence or absence of contaminating materials, indicating a direct effect of the energy. The changes are influenced slightly by the temperature of the virus during irradiation.

Inactivation curves for different viruses, bacteria and single cells of higher animals will be presented. These show slope constants which are strikingly different and which can be related to the size of the organism.

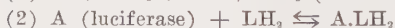
By utilizing the quantum concept for the absorption of photo-electrons of known path lengths we may calculate the size of the vital spot within these organisms. The resulting values are in surprising agreement with like determinations utilizing other biological considerations. The bearing of these results on gene size, evolution of cell size, etc., will be discussed.

26. THE SIGNIFICANCE OF THE 'FLASH' OF LUMINESCENCE FOLLOWING ANAEROBIC OXIDATION OF LUMINOUS BACTERIA. Frank H. Johnson and K. L. van Schouwenburg. Princeton University and the Biophysical Research Group, Utrecht, Holland. (Lantern; 15 min.)

With the aid of a photo-electric cell-galvanometer apparatus, a study of the 'flash,' or excess luminescence which occurs when oxygen is suddenly admitted to a suspension of luminous bacteria, previously dim or dark through oxygen lack, has provided new evidence concerning the light-emitting and associated reactions.

The luciferin appears to act as a H-carrier, capable of a two-way oxidation: (1) a reversible dark oxidation, and (2) a (probably irreversible) luminescent oxidation by luciferase and oxygen. Cyanide has no direct effect on the luciferin-luciferase system in bacteria although urethane reduces luminescence intensity, evidently by acting on the luciferase. In all these respects the light emitting system in bacteria resembles that in Cypridina extracts.

In the absence of oxygen, bacterial luciferin comes into an equilibrium which may be greatly influenced by glucose and certain other H-donators. The following scheme is proposed to represent the reactions in the phenomena observed.



The L_1 represents the oxidation product of luciferin (LH_2) in the luminescent reaction. If no substrate is present that can be anaerobically metabolized, the luciferase appears to undergo a reversible proteolysis in the absence of oxygen, represented by a fourth equation:



in which nB denotes the proteolytic decomposition products.

27. CHANGES IN THE ABSORPTION SPECTRUM OF LUCIFERIN SOLUTIONS. Aurin M. Chase, Princeton University. (Introduced by E. N. Harvey.) (Lantern; 15 min.)

The visible absorption spectrum of solutions of freshly dissolved Cypridina luciferin, partially purified by Anderson's method, has a progressive rise toward the shorter wavelengths, with no perceptible maximum. During spontaneous non-luminescent oxidation a maximum appears at about $470\text{ m}\mu$, indicating a shift in absorption to this region from the ultra-violet. Subsequently this maximum disappears, accompanied by a decrease in absorption throughout most of the visible

spectrum. These changes are much more pronounced and their rate is much greater in neutral aqueous solutions of luciferin than in n-butyl alcohol solutions. The rate of oxidation of luciferin is also greater in neutral aqueous solution than in butyl alcohol.

R. S. Anderson has reported that when luciferase is added to freshly dissolved luciferin luminescence occurs more rapidly than when the luciferin has stood in solution exposed to air before adding the enzyme. Two distinct types of reaction are indicated. On the basis of this he has postulated a reversibly oxidized compound formed from luciferin during non-luminescent oxidation. The initial change in the absorption spectrum of luciferin solutions is believed to represent the formation of this compound. The subsequent decrease in the absorption spectrum may represent a further reaction, possibly an oxidation.

A change in the absorption spectrum of luciferin solutions is also observed as a result of the luminescent reaction in the presence of luciferase, but it has not been possible to study the course of this change because the luminescence interferes with measurements of light absorption.

28. REVERSIBLE DEPRESSION OF THE PROTOPLASMIC E.M.F. OF VALONIA BY CYANIDE AND ETHER. Gordon Marsh, State University of Iowa. (Introduced by Eleanor H. Slifer.) (Lantern; 15 min.)

The steady dark potential of *Valonia ventricosa* is reversibly depressed by KCN (or NaCN) between 2×10^{-8} and 5×10^{-3} M. Low concentrations are relatively more effective; the maximum depression (40 to 75%) is practically reached at 1×10^{-4} M. Recovery of the original potential upon removal to sea water is slower the higher the concentration of cyanide used. In the light the depression of the steady potential is greater for all concentrations, both as an absolute and as a percentage amount; at 1×10^{-3} M the effect of light on the E.M.F. has virtually disappeared. The light response for cells whose potential is depressed by cyanide in the dark is normal in form but reduced in magnitude.

A similar effect is produced by ether between 0.01% and 1.0% by volume; 1.5% and higher concentrations coagulate the protoplast. Between 0.5% and 1.0% ether causes an increase in potential (stimulation) to around eight times the magnitude of the normal dark potential, requiring about 3 hours to descend to a steady level (narcosis). Illumination during the stimulation phase results in an irregular light response, the potential oscillating continuously about the stimulated dark level. The light response during the narcotic phase is similar to that of cells depressed by cyanide.

The results are in harmony with the conclusion that the electromotively active materials are formed in the complex of respiration and photosynthesis, as postulated in Lund's oxidation-reduction theory of bioelectric currents.

29. THE ELECTRICAL POTENTIAL OF FROG SKIN. T. Cunliffe Barnes, W. J. Coe and H. L. Golubock, Yale University. (Lantern; 8 min.)

Pressure and tension depress the potential of frog skin. Diphasic electrical waves produced by tapping isolated skin in Ringer's solution were recorded with an oscillograph. Most of the spike potentials were 0.5 to 5 millivolts and lasted 0.01 to 0.05 seconds.

Skins arranged in contact with outside against inside do not show summation of e.m.f. Skin preparations of small area (0.5 cm. diameter) usually have lower potentials than larger pieces (1.5 cm. diameter) owing to the presence of spots of high potential eliminated on the smaller skins.

Puncturing the skins with a needle does not reduce the potential to zero. With successive punctures the slight falls in potential are proportional to the magnitude of the potential before puncturing. The results indicate a continuous process maintaining potential against the short circuits through the skin.

The temperature characteristics for skin potential are 5,000, 10,000, and 17,000 calories.

The oxygen consumption of skins was measured in Fenn respirometers after the potential was taken, but no correlation between the magnitude of the potential and O_2 uptake was found. Electrodes were introduced into the respirometer flask and eosin added by tipping. In 93.3% of the cases the potential increased and in 66.6% the O_2 uptake also increased. In untreated skins slight fluctuations in potential followed slight changes in O_2 uptake ('fringe effect') but the total potential is not related to total O_2 consumption.

30. ANTAGONISM BETWEEN HEAVY WATER AND ADRENALIN ON THE RATE AND ACTION CURRENT OF THE HEART OF THE TURTLE. T. Cunliffe Barnes, Yale University. (Lantern; 7 min.)

The action current in the turtle's auricle was recorded with a Matthews oscillograph before and after treatment with Ringer's solution containing 15 to 99% heavy water and 1 in 50,000 and 1 in 50,000,000 parts of adrenalin. The heavy water depressed the strength of contraction, rate of pulsation, and action current in the order named. These effects were antagonized by adrenalin. In 60% D_2O , 1 in 50 million parts of adrenalin usually increased the rate of pulsation. In one case the action current was increased from 2.6 to 8.4 mv. by this treatment. In another heart in 99% D_2O + 1 in 5 million adrenalin the action current was depressed from 7.5 to 3.7 mv. Hearts arrested in 60 and 99% D_2O were revived by 1 in 50 million parts of adrenalin. No potentiation of adrenalin and D_2O was indicated. The oxygen consumption of ventricular strips from the same heart in Fenn respirometers was not greatly affected by 60 and 99% D_2O over an hour's run.

31. THE RELATION BETWEEN 'SPONTANEOUS' ACTIVITY AND SYNAPTIC TRANSMISSION IN THE NERVOUS SYSTEM OF THE CRAYFISH. C. Ladd Prosser, University of Illinois. (Lantern; 15 min.)

Isolated abdominal ganglia of the crayfish show 'spontaneous' activity which can be analysed into repetitive discharge in single neurones. Upon the 'spontaneous' background transmission across single synaptic junctions was studied in the last abdominal ganglion. Since summation is necessary the number of efferent units excited by a given afferent volley is a measure of synaptic excitation. Acetylcholine, physostigmine and prostigmin have no potentiating action and do not stimulate the nerve cells to discharge unless in toxic concentrations. Acetylcholine is apparently not involved in synaptic transmission in this animal.

If the ganglia are bathed with saline containing less potassium than the blood the number of units firing 'spontaneously' is markedly increased. Higher concentrations of potassium depress the 'spontaneous' activity by reducing the number of active units. The synaptic response is enhanced by those potassium concentrations which increase the 'spontaneous' activity and is depressed by potassium concentrations which diminish spontaneity. These potassium effects are not antagonized by calcium but the stimulating action of low potassium can be overcome by adding rubidium to the solution. Increase in intracellular acidity by CO_2 enhances activity and decrease in intracellular acidity depresses activity. The pH effects can be explained by an increase and decrease in intracellular free potassium. It is concluded that whether or not a neurone discharges 'spontaneously' or is excited synaptically depends upon the ratio of the potassium inside to that outside.

32. THE INFLUENCE OF POTASSIUM ON THE SENSITIVITY OF INTESTINAL MUSCLE TO ACETYLCHOLINE. John L. Fuller and H. Michael Kroll, University of Maine. (Lantern; 10 min.)

The concentration of acetylcholine necessary to produce contraction of the intestinal muscle of the guinea pig has been determined in Ringer-Dale solution with reduced and augmented K content. The threshold in the standard solution is at a concentration of about 10^{-10} , and does not change greatly when K is reduced to 0.5 standard concentration or increased to 1.5 standard. When the K content is doubled the threshold is increased more than tenfold, and when it is reduced to 0.1 standard the threshold is decreased to a lesser degree.

Rhythmically contracting muscle has its tonus increased by a sudden increase of 10% in the K concentration. Following this it responds to acetylcholine, though the threshold is raised. However, muscle which has been recently stimulated by acetylcholine does not respond to a 10% increase in K. That the response to K is not due to its causing the liberation of acetylcholine by the myenteric plexus is shown by the fact that atropine does not abolish the response.

A tentative explanation is that acetylcholine stimulates the neurons of the myenteric plexus, and that K is mobilized thus stimulating the muscle. In high K solutions not enough K is produced to cause a critical change in the K concentration at the myoneural junction, but response will occur if more acetylcholine is added. Sudden addition of K to the medium stimulates in the same manner as liberation of K by the nerve ending.

33. THE EFFECT OF SMOOTH MUSCLE STIMULANTS ON THE VASODILATOR ACTION OF ACETYLCHOLINE. Theodore Koppanyi, Georgetown University, School of Medicine. (Lantern; 15 min.)

In dogs anesthetized with ether or nembutal, the vasodilator effects from small intravenous doses of acetylcholine (0.0005 to 0.005 mg. per kg.) and pilocarpine (0.005 to 0.01 mg. per kg.) have been determined. Following these control injections, large amounts of pilocarpine were given in divided doses (0.25 to 2.0 mg. per kg.). As a result of the administration of 0.25 to 0.5 mg. per kg. of pilocarpine, the vasodilator actions of 0.0005 to 0.005 mg. of acetylcholine and of 0.005 to 0.01 mg. of pilocarpine were, as a rule, entirely abolished, and after 1 to 2 mg. of pilocarpine, even the effects of 0.1 to 0.5 mg. of acetyl-

choline were abolished or greatly reduced. Under the full effects of pilocarpine, dogs were able to survive, with a small and rather temporary fall of blood pressure, the effects of 0.5 to 5.0 mg. per kg. of acetylcholine.

It was postulated that this remarkable effect of large doses of pilocarpine is due to a direct smooth muscle stimulant action preventing relaxation of the contracted arteriolar musculature. To test this postulate, typical smooth muscle stimulants: pitressin and barium chloride were administered instead of pilocarpine. Both of these drugs antagonised the vasodilator effects of acetylcholine. With barium chloride (4 to 20 mg. per kg.) it is possible to abolish or to reduce the vasodilator effects from 0.0005 to 1.0 mg. per kg. of acetylcholine, and the larger doses of acetylcholine (0.5 to 1.0 mg. per kg.) may produce in the presence of effective concentrations of barium their nicotine-like pressor effects.

The action of smooth muscle stimulant drugs to antagonise the effects of cholinergic vasodilator substances will be used extensively in the study of pharmacodynamics of the autonomic nervous system.

34. FREE AMINO ACID IN NERVE AXOPLASM. Robert H. Silber and Francis O. Schmitt, Washington University. (10 min.)

In squid axoplasm, cations are present in a concentration four to five times that of the inorganic anions (chiefly chloride). A similar situation exists in lobster nerve and a considerable fraction of the organic anions making up the deficiency appears in deproteinized extracts of lobster nerve. High non-protein nitrogen and formol titration values for such extracts, as well as the fact that the equivalent weight of the anion must be of the order of 100, suggested that the anion may be a free amino acid (Bear and Schmitt; Schmitt, Bear, and Silber).

Large quantities of lobster nerve extract were deproteinized with alcohol at 2°C. Analysis was made of the solid obtained by evaporation of this extract. Amino and carboxyl groups were determined by nitrous acid and ninhydrin manometric methods. The anion deficit in the deproteinized preparation (approximately half that of whole nerve) was found to be very nearly equal to the number of equivalents of amino groups. Electroneutrality could thus be realized if the substance supplying the anions is a dicarboxylic amino acid. The fact that a corresponding excess of carboxyl over amino groups was not obtained in the ninhydrin analyses does not disprove this assumption. The extract yielded a barium salt from alcoholic solution, which may be considered further support of the view that this substance is a dicarboxylic amino acid. That this substance may be of considerable importance in nerve is indicated by the fact that its concentration may be estimated conservatively at 2000 mg. per cent.

35. ROLE OF THE AURICLES IN VENTRICULAR FILLING IN THE TORTOISE. Stephen Wood Gray and F. R. Steggerda, Department of Physiology, University of Illinois. (Lantern; 6 min.)

Using a cinematographic method previously reported (1938), the effect of the auricular systole upon the ventricular volume curve in forty tortoises was investigated. Pictures were made both with and without the pericardium. By means of ligation of the auricles in such a manner that the blood flow was not impeded, the auricular effect was isolated from other ventricular changes. Measurements in the regularly beating heart showed that the auricular contribution was at a

minimum (7%) at a heart rate of 8 beats per minute. The auricular contribution increased to about 40% as the rate was decreased to 3 per minute and also as it was increased to 25 per minute. This would seem to indicate that the auricle is of greater importance at extremely slow or extremely rapid heart rates and that it is possible to consider it in the light of a mechanism for the protection of the ventricle.

36. FUNCTIONAL RESULTS OF MUSCLE TRANSPLANTATION IN THE HIND LIMB OF THE ALBINO RAT. Roger W. Sperry, University of Chicago. (Introduced by Paul A. Weiss.) (Lantern; 10 min.)

Muscle transpositions in the hind limb of the albino rat were performed so that contraction of the main dorsi-flexor muscles of the foot (m. tibialis anterior, m. extensor digitorum longus) produced plantar flexion instead of dorsi-flexion, and contraction of the lateral gastrocnemius muscle, normally a plantar flexion of the foot, produced dorsi-flexion instead of plantar flexion. All other shank muscles were removed. Nine rats (seven unilateral and two bilateral cases) exhibited both in 'spontaneous' and reflex activity complete reversal of all plantar and dorsi-flexor phases of the movements of the operated limbs. This reversal persisted for more than 10 months with no functional adjustment either in common activities or in special trained performances demanding a single elementary foot movement.

Analysis showed that the reversal was due to clear-cut reciprocal contraction and relaxation of the transposed muscles; that mechanically the transposed muscles were capable of producing normal movement; and that the sensitivity of the shank, ankle, foot, and transposed muscles had not been impaired by the operation. Amputation of contralateral foot, amputation of front legs, training the rats to rise on hind limbs as well as to climb a 45 cm. ladder for food, failed to result in reëducation of the foot movements.

This unmodifiability of the contraction patterns in the hind limb musculature of the rat compares more closely with conditions found in amphibians than with those reported for higher mammals.

See also demonstration number 151.

37. CHANGE OF STATE OF THE ISOLATED SINGLE MUSCLE FIBER AT A CRITICAL LENGTH. Robert W. Ramsey and Sibyl F. Street, Department of Physiology, University of Rochester. (Lantern; 15 min.)

In a previous investigation we have shown that 1) the resting tension of an isolated muscle fiber of the frog is due to the sarcolemma, 2) the tension developed in a maintained tetanus is a maximum at the resting length (R.L.), decreases with stretch or shortening, and is perfectly reversible over a range of lengths from 70% R.L. to 200% R.L.; 3) that striking permanent changes in the properties of the fiber occur if it is allowed to shorten below 60-70% R.L., the most marked of which is that the fiber remains shortened after cessation of the stimulus at whatever length it was allowed to shorten to. This and other changes in properties, brought about by shortening a muscle fiber to a critical length, are further investigated with respect to tension developed in a maintained tetanus at various lengths, rate of development of tension, excitability, etc., and these physiological changes are correlated with concomitant changes in structure of the fibers.

38. THE ABSORPTION OF CARBOHYDRATES FROM THE GASTRO-INTESTINAL TRACT OF BROOK TROUT, *SALVELINUS FONTINALIS*. Arthur M. Phillips, Jr., N. Y. State Conservation Department and C. M. McCay, Cornell University. (Lantern; 15 min.)

Sucrose absorption studies were conducted on brook trout fingerlings weighing from 6 to 14 gm. each. All food was placed in number five gelatin capsules and inserted into the stomach by means of fine pointed forceps. The trout were divided into groups of three and each group held in a separate well aerated aquarium. At intervals of 3, 6, 9, 12, 24, 48 and 72 hours, one group was killed, the gastro-intestinal tract removed, and analyses were run upon the tracts and the water of the aquarium in which the fish were held. These experiments were run in triplicate.

The percentage absorption for a given time interval is the same when either 10, 25 or 50 mg. of sugar are fed. Thus the rate of absorption increases with an increase of sucrose concentration in the gastro-intestinal tract. These results are inconsistent with those obtained by Cori in his studies on rats. In no case was it possible to show an excretion of glucose from the trout even when so large an amount as 100 mg. per fish was fed. Trout seem able to digest and absorb large amounts of sucrose in terms of body weight.

Preliminary studies indicate that when sucrose is coated with 25% tallow, the digestion and absorption of the sugar is retarded for a period of about 12 hours, after which time percentage absorption increases rapidly. The addition of hog spleen to the tallow-sucrose mixture, enables the trout to remove the tallow coating in a much shorter period and absorption is more rapid. This is probably due to a softer and more easily penetrated mass caused by the juices of the meat. The addition of dry casein to the tallow-sucrose mixture had no such effect.

Although these experiments show trout are able to absorb large amounts of sucrose, experiments in progress at the Cortland Experimental Hatchery indicate that trout fed high levels of sucrose for long periods do not make good growth and may develop large and swollen livers, of abnormal color.

39. THE SECRETION OF PHENOL RED BY THE FROG RENAL TUBULE. Roy Ph. Forster, Dartmouth College. (Introduced by W. B. Unger.) (Lantern; 15 min.)

In a previous report it was demonstrated that inulin and creatinine are excreted by the frog kidney solely by the process of ultra-filtration at the glomerulus, neither substance being secreted nor reabsorbed by the renal tubule cells. In this study of 70 clearance periods in ten bullfrogs, inulin was used as a measure of the glomerular filtrate, and its clearances were compared with the simultaneous clearances of phenol red over wide plasma concentrations of the latter, ranging from 0.15 to 95 mg. per cent. The phenol red fraction bound to plasma proteins, and therefore unavailable for ultrafiltration at the glomerulus, was determined at various total phenol red plasma concentrations.

Unlike the creatinine/inulin clearance ratio which remained 1.0 despite the plasma concentration of either substance, the phenol red/inulin clearance ratios varied from 10.0 at the lowest phenol red plasma concentrations to 0.62 at the highest levels. The phenol red/inulin clearance ratio at phenol red plasma concentrations below 10 mg. per cent had a mean value of about 3.0.

The curvilinear relationship between phenol red/inulin clearance and total phenol red plasma concentration is interpreted as indicating a tubular secretory maximum for phenol red reached at low plasma levels. Tubular secretion accounts for the greater amount of phenol red excreted at low plasma phenol red concentrations, while at higher concentrations, due to the tubular maximum, glomerular filtration is predominant over tubular secretion. The phenol red/inulin clearance ratios of less than 1.0 when the total plasma phenol red concentrations are over 30 mg. per cent emphasize the unavailability of bound phenol red for filtration.

40. THE EFFECTS OF INCREASED VASCULARIZATION AND THYROXIN ON HAIR GROWTH IN UNDERFED ALBINO RATS. Earl O. Butcher, Hamilton College. (Lantern; 10 min.)

When increased vascularization and edema of the skin of underfed rats were created by daily external applications of cantharides, capsicum, xylene, or benzoic acid, the quiescent hair follicles became active and produced hair externally 10 to 14 days after the beginning of the applications. Daily subcutaneous injections of 0.5 cc. of 0.3% histamine solution were equally as effective.

Subcutaneous injections of 0.2 mg. of thyroxin daily for 3 days in underfed rats caused the hair to appear externally about 9 days after the initial injection. External applications of irritants simultaneously with injections of thyroxin induced hair growth usually a day sooner than the thyroxin alone.

By dissolving thyroxin in the irritants and applying them daily for many days, hair appeared externally about 10 days after the first application.

When hair follicles are inactive because of the lack of nutrition, increased vascularization by irritants will, therefore, often supply the follicle with substances necessary for growth. The irritation, however, must be severe and continued for several consecutive days to be effective. These experiments also show how very effective thyroxin is in inducing activity in hair follicles which are not well nourished, and that thyroxin can be incorporated in the irritant and absorbed through the epidermis.

41. EFFECT OF EXTRACTS OF URINE FROM PERNICIOUS ANEMIA AND GASTRIC CANCER PATIENTS ON GASTRIC SECRETION. M. H. F. Friedman, D. J. Sandweiss, R. O. Recknagel and T. L. Patterson, Department of Physiology, Wayne University College of Medicine. (Lantern; 10 min.)

Extracts have been prepared from the urine of healthy individuals and of pernicious anemia and gastric cancer patients. When administered intravenously to dogs with gastric fistula, all three types of urine extracts depress greatly the secretion of gastric juice stimulated by histamine. The inhibition of secretion would appear to be due to a specific principle in the urine but no evidence is yet available to indicate by what organ of the body it is elaborated. A study is being made to determine if the degree of gastric secretory inhibition can be correlated with the extent of gastric pathology or degree of anacidity.

42. AGE DIFFERENCES IN RESISTANCE TO CO POISONING AMONG YOUNG RODENTS. John A. Cameron, University of Missouri. (Lantern; 10 min.)

Rats, rabbits, and guinea pigs, aged 1 day to 1 year, were exposed to an atmosphere of 95% CO and 5% O₂. All the guinea pigs died after 4 to 4.5 minutes exposure. Rabbits showed a high resistance at birth (40+ minutes) which dropped abruptly to 20 minutes on the 6th day and to 6 minutes on the 14th day. Adults survived 4 to 4.5 minutes.

Albino rats survived 27+ minutes at 1 day, 16 to 20 minutes from 2 to 7 days. 6 to 7 minutes from 8 to 9 days, 4 minutes from 10 to 16 days and 2 minutes after 16 days of age. This behavior is similar to that reported by Avery and Johlin for mice.

It is suggested that the abrupt changes in resistance may indicate shifts from the anaerobic juvenile type of respiration described by Enzmann and Pineus toward the adult type. Rats, born after 21 days gestation, undergo five such shifts after birth, while the 30-day gestation of the rabbit leaves only the last three of these shifts to be accomplished. Young guinea pigs may be thought to have completed the development of adult respiratory process during their 65-day gestation. This concept is supported by the observation that the whole graph of the course of declining resistance in the rabbit resembles that of the rat from the 6th day on. It should also be noted that relief of CO poisoning by irradiation has been successful only with rats over 1 week old, the poisoning in younger animals proving irreversible

43. FURTHER STUDIES ON THE NATURE AND ACTION OF DIANTLIN, A HORMONE-LIKE SUBSTANCE CARRIED ON THE SPERMATOOZOA OF THE OYSTER. J. B. Allison and T. C. Nelson, Rutgers University. (Lantern; 15 min.)

Some of the effects and properties of a substance, called diantlin, which increases the flow of water through the oyster have been reported by Nelson '35, '36, and Nelson and Allison '37 and '38. In recent work the spermatozoa have been separated into a basic protein and a nucleoprotein fraction. The basic protein was prepared by suspending spermatozoa, previously fragmented by cold ethyl alcohol or by freezing, in cold 0.1 N HCl for 24 to 48 hours. The basic protein was precipitated from the filtered acid solution by the addition of 0.1 N NaOH until the solution was approximately pH of 10.3. The nucleoprotein fraction was prepared by suspending the acid insoluble fragments in cold 0.1 N NaOH for 18 hours. The nucleoprotein was precipitated by adding 0.1 N HCl to the filtered alkaline solution until the solution was approximately pH of 4.4. The basic protein, neutralized and dissolved in sea water had little or no effect upon the rate of water flow through the oyster. The nucleoprotein fraction, neutralized and dissolved in sea water, caused a transitory increase in the flow of water through oysters most susceptible to stimulation by spermatozoa. When approximately neutral solutions of these two fractions were combined a precipitate formed. This compound protein precipitate, suspended in sea water, was effective in stimulating an increase of water flow. Benzene soluble fractions also were prepared from spermatozoa. These fractions, which have been shown by Galtsoff ('38) to stimulate spawning in the oyster, did not increase the flow of water.

44. GONADAL STIMULATION IN SEXUALLY IMMATURE FUNDULUS BY IMPLANTATION OF ADULT HYPOPHYSES. Samuel A. Matthews, Williams College. (Lantern; 10 min.)

The pituitary glands of sexually mature individuals of *Fundulus heteroclitus* were removed and implanted into sexually immature animals. Since the sexes of the young animals could not be distinguished externally, implanted pituitary glands were taken from adults of both sexes at random. Two methods of implanting were employed. When a single gland was placed in the anterior chamber of the eye only indifferent results were obtained. Subsequent histological examination showed that the implanted pituitary gland in nearly all cases was shrunken and contained only small cells which were obviously inactive. A preliminary experiment then showed that an adult pituitary gland placed in the abdominal cavity of a young animal could be recovered at the end of 4 or 5 days. Hence the second method used consisted of serial implants into the abdominal cavity at intervals of 3 days in order to provide a continuous supply of whatever hormones might be present in the adult gland. After 4 weeks every young male so treated showed very active testes while control animals kept in the same tanks were still inactive. In the females the changes were not as striking, nevertheless in all cases an enlargement of the ovary and of its follicles was evident.

45. THE EFFECT OF TESTOSTERONE PROPIONATE INJECTIONS INTO CASTRATE MALE FROGS, *RANA PIPIENS*.¹ Opal M. Wolf, Goucher College. (Lantern; 15 min.)

Following intra-muscular injections of a total of 38 mg. of testosterone propionate² in sesame oil into adult castrate male frogs for 19 days, the vestigial oviducts showed marked enlargement, particularly in the uteri and adjacent oviducts. Wolf, 1928, reported that the vestigial oviducts of castrate male frogs did not enlarge following castration. The thumbpads of the injected males showed marked development of the mucous glands when killed in late August and the epidermis was beginning to be thrown into ridges due to the mitotic activity of the epidermal cells (the latter were not as marked as in the normal controls). Pigment cells had migrated into the epidermis from the dermis.

These experimental animals were castrated early in July when the mucous glands were atrophied and the epidermis of the thumb-pad was smooth and showed a scarcity of pigment cells. The control castrates in late August showed this condition.

Males injected for a comparable time with a total of 2800 rat units of dihydroxyestrin² in the form of alpha-estradiol benzoate have not shown enlargement of the oviducts. It appears from these experiments that the oviducts of adult male castrates are more sensitive to testosterone propionate than to dihydroxyestrin.

Amplexus did not occur when these injected castrate males were placed with castrate females injected with dihydroxyestrin. In late August the injection of 5 anterior pituitary lobes from *Rana pipiens* for 2 successive days did not evoke amplexus.

¹ The experimental work has been carried on at the Marine Biological Laboratory at Woods Hole and Goucher College.

² These preparations have been supplied by the Schering Corporation through the courtesy of Dr. Max Gilbert.

46. RUDIMENTS OF EPIDIDYMS AND VAS EFERENS IN FEMALE TURTLES. Llewellyn T. Evans, University of Missouri. (Lantern; 10 min.)

Three out of 24 adult turtles (*Chrysemys picta bellii*) were found to have rudiments of epididymis and vas eferens while a fourth female had a small rudiment of vas eferens only. These rudiments were in close proximity to the kidney-adrenal complex and occurred in loose connective tissue.

Two of the 4 females had received injections of anterior pituitary extract, 20 and 24 daily injections, respectively (0.5 gr., 0.6 gr., total dry weight); the other two remained uninjected.

The epididymis in the two injected was distended and the cells were high cuboidal in shape while that of one of the uninjected was smaller in diameter and less regular in shape with relatively low cuboidal cells in its epithelial wall.

The nuclei of vas eferens and epididymis were normal in appearance in the injected females but irregular and somewhat pyknotic in appearance, staining very heavily with haematoxylin, in uninjected females.

Recent work shows that theelin causes some hypertrophy in the vas deferens and epididymis in male *Anolis carolinensis* even though testicular atrophy occurs. It is suggested that the pituitary injections stimulated the turtle ovary to increase its output of theelin which in turn had a slight stimulating effect upon the rudiments of the vas eferens and epididymis present. There are several reports of adult male reptiles with well developed oviducts but this is believed to be the first concerning adult female reptiles with rudimentary male genital structures.

47. THE DISTRIBUTION OF INTERMEDIN: A NEW BIOLOGICAL METHOD OF ASSAY WITH TESTS UNDER NORMAL AND EXPERIMENTAL CONDITIONS. L. H. Kleinholz and H. Rahn, Harvard University. (Lantern; 15 min.)

Quantitative studies in the distribution of intermedin in the pituitary glands of several vertebrates have been made, the hypophysectomized lizard, *Anolis carolinensis*, serving as the test animal. An *Anolis* unit for intermedin is defined as that weight of pituitary powder which, when injected in the form of neutralized NaOH-extract into each of 10 hypophysectomized *Anolis* (each measuring between 5-6 cm. from tip of snout to anus) will elicit an average stage 1 response on a scale dividing the two extremes of the color range into 5 stages. The anterior lobe of the chicken's hypophysis contains large amounts of intermedin. The cephalic-third of this lobe contains 20 times more intermedin than the caudal-third. The same region is also more potent than equal weights of pars intermedia tissue from beef. The amounts of intermedin in the glands of light-adapted frogs and of frogs kept in darkness do not differ significantly.

48. HORMONES IN THE DEVELOPMENT OF THE CHICK. Nicholas W. Fugo, State University of Iowa. (Introduced by F. A. Stromsten.) (Lantern; 15 min.)

The pituitary primordia of 33-38 hour embryos were removed by surgical means (Fugo and Witschi, 1938). The further development proves in general that all embryonic differentiation is independent of the pituitary, but later the lack of growth hormone and thyrotropic hormone results in plainly visible deficiencies.

It also seems that abnormalities of testicular and ovarian development are due to the absence of gonadotropic hormones.

The experimental animals are smaller than controls but show normal body proportions. The down is more darkly colored, showing many pigment granules and small air spaces. The thyroids fail to enlarge, having small undeveloped follicles widely separated by blood vessels and containing only minute amounts of colloid. Thyrotropic hormone rectifies the condition of the down and thyroid structure but exerts no appreciable influence on body size.

The testes do not develop normal amounts of intertubular material and show small under-developed seminiferous tubules. The left ovary is deficient in cortical development. The right ovary is apparently unaffected. Since the gonoducts are found in a normal condition in the absence of the pituitary, their sexual differentiation does not seem to be under hormonal control. It is doubtful if the gonads of experimental animals release appreciable amounts of sex hormones.

Hypophysectomized animals fail to withdraw the yolk sac within the body cavity even though living in the egg up to 8 days beyond the normal incubation period. Operated animals with the pituitaries left intact absorb the yolk sac at the proper time.

49. THE EFFECTS OF EMBRYONIC ESTROGENIC TREATMENT UPON SEXUAL CHARACTERS OF ADULT BROWN LEGHORN COCK. L. V. Domm, University of Chicago. (Lantern; 15 min.)

Brown Leghorn eggs received single injections of oestrin between 3rd and 5th days of incubation and treated embryos were allowed to hatch and mature. Estrogens used were progynon-B (1500 to 3000 R.U.) and theelin (0.5 to 1.0 mg.). A total of 410 eggs were treated hatching 69 chicks. Of these 51 (24 male and 27 female) developed beyond period of sexual differentiation and form the basis of this study.

Males underwent significant sexual transformations. Sexes were invariably distinguishable at time of sexual differentiation on basis of external characterization. Certain males were seemingly normal while others revealed early feminizing effects in plumage but none revealed complete female plumage during first year though this occurred in recent experiments. However, following the first molt certain individuals assumed complete female plumage whereas others showed no change. Observations indicate that female plumage may be retained following the second yearly molt. Fluctuations in plumage characterization occurred. Head furnishings were generally masculine. Crowing and treading were observed though sexual libido appeared less intense than in normals.

Necropsy revealed significant changes in reproductive system. The left gonad generally smaller than normal is flattened and ovary-like. Modifications of right are similar though less extreme. Various developed oviducts occur though in some they are entirely absent.

Histology of gonads in extreme cases shows left is ovotestis with irregular surface cortex underlying which are spermatogenic tubules. Various sized, scattered, follicles with normal ova showing yolk granules and nuclei have been found in cortical component. Modifications of right are of same order though less extreme and no follicles have been found.

50. QUANTITATIVE DETERMINATION OF THE FOLLICLE STIMULATING AND THE LUTEINIZING HORMONES OF THE HYPOPHYSIS. Emil Witschi, State University of Iowa. (Lantern. 15 min.)

Biological tests separating the effects of luteinizing and follicle stimulating gonadotropic hormones are mostly unsatisfactory because the indicator organs respond, directly or indirectly, to both principles. It was therefore a welcome discovery when it was found that regenerating feathers of African Weaver Finches react directly and exclusively to the luteinizing hormone only. This makes it possible to calculate for any preparation containing hypophyseal hormones a rat: finch quotient which essentially expresses the FSH:LH ratio. Evidence will be presented to prove that the human pituitary is richer in FSH than any animal glands, while those of beef, whale, turkey and salmon are extremely rich in LH. The possibility of vicarious functions of the two hormones will be discussed.

51. ON THE MECHANISM AND HORMONES CONCERNED IN INCREASE OF BLOOD FAT IN BIRDS. Oscar Riddle and Tellef Senum. (Lantern. 15 min.)

Increase in blood fat (fowl) associated with ovarian activity has been recently and more definitely identified with ovarian output of estrogenic hormone. It is here shown that estrogen does not act through the pituitary, since estrone and dihydroestrone exert their full action in immature pigeons deprived of anterior and posterior lobes. The pancreas is likewise found not essential since estrone greatly accentuates the lipemia which follows pancreatectomy.

Though relatively large doses of androgens produce no lipemia such an action (small) is exerted by desoxycorticosterone acetate.

The extent to which the fat (exclusive of phospholipid) of whole blood is increased by normal estrogen production incident to growth of a pair of ova has been measured and found to differ notably in doves and pigeons. This value usually reaches 600 mg. per cent in doves; it attains 1100 mg. per cent in pigeons. In both species blood fat begins to increase approximately coincident with beginning rapid growth in an ovum, attains a maximum at or before the ovulation of this ovum, then rapidly falls to original value. The curve for blood fat thus parallels that for the blood calcium which one of us earlier found to be regulated by estrogen.

In doves and pigeons (not in rabbits) lipemia of pituitary origin is traceable to gonadotropin and its action through the ovary; it is effective in females only, and is effective in pituitaryless females. Prolonged tests with both gonadotropin and estrogen show unexplained alternating periods of effectiveness and ineffectiveness of both types of hormone.

52. LIMITATIONS OF FOOD AS A FACTOR INFLUENCING ENDOCRINE REACTIONS IN THE CHICK. W. R. Breneman, Waterman Institute, Indiana University. (Lantern; 15 min.)

Single comb White Leghorn cockerels and pullets were given food only on alternate days and were compared with control chicks permitted to feed at will. Examination of chicks up to 30 days after hatching indicated that secretion of pituitary gonadotropin was suppressed. Injection of androgen (dihydroandroster-

one-benzoate) into chicks on limited diet elicited greater proportional comb growth than did the same amount given to chicks on normal diet. The difference in response to male hormone observed between cockerel and pullet on normal diet was not present in the limited diet birds. Injection of hog anterior pituitary extract also produced a greater proportional growth of testes and comb in the latter series. A study was made of the variation in the two groups of chicks and it was noted that the untreated limited diet birds were very uniform, but when injected were nearly as variable as the normal diet animals. Injections made daily for 10 days, from the fifth to the fourteenth days, proved to be much more effective than did injections over a 5-day period, from the fifth to ninth days. A total of 386 chicks were used.

53. (No abstract received.)

54. (No abstract received.)

55. LIGHT AND REPRODUCTION IN PHEASANTS. I. EFFECT OF INTENSITY OF ARTIFICIAL ILLUMINATION ON THE REPRODUCTIVE CYCLE. Leonard B. Clark, Union College, S. E. Leonard, Rutgers University and Gardiner Bump, New York State Conservation Department. (Lantern; $7\frac{1}{2}$ min.)

Ring-necked pheasants were illuminated continuously at intensities of 106, 5, 0.5, and 0.05 foot candles with artificial light. Natural daylight was rigorously excluded. The experiment was started on November 24 and 34 days later the birds at 106 foot candles began laying. The laying rate was computed as eggs per day per hen averaged for 10-day periods and gave values of 0.4, 0.2, 0.225 and 0.01 for the successive 10-day periods. Egg laying ceased on the fifty-ninth day of the experiment. At 5 foot candles, pheasants began laying on the forty-first day with laying rates of 0.1, 0.2, 0.55 and 0.7 for successive 10-day periods. Birds illuminated with 0.5 foot candles laid a total of one egg on the fiftieth day although the ovaries increased to the same size as in females exposed to 5 foot candles. Pheasants exposed to 0.05 foot candles and controls under natural daylight showed no gonad development nor were eggs laid. It is concluded that the threshold intensity for stimulation of the gonads is lower than that necessary for egg laying. As far as could be determined by weight, rate of increase in size was independent of intensities above the threshold while rate and duration of egg laying was dependent on the intensity.

56. LIGHT AND REPRODUCTION IN PHEASANTS. II. EFFECT OF RATE OF INCREASE OF DAILY ILLUMINATION ON THE REPRODUCTIVE CYCLE. Leonard B. Clark, Union College and Gardiner Bump, New York State Conservation Department. (Lantern; $7\frac{1}{2}$ min.)

In the latitude of Schenectady sunlight increases by an average of 2 minutes, 10 seconds per day from mid December to mid June with a maximum of about $15\frac{1}{2}$ hours. Beginning with $10\frac{1}{2}$ hours illumination of 5.0 foot candles, groups of pheasants received daily increments of 360, 30, 15, and 5 minutes until they were receiving $15\frac{1}{2}$ hours per day. By computing the efficiency of stimulation by the ratio of eggs to number of hen days in each group, those receiving the total incre-

ment at once gave a value of 0.0714, while those receiving daily increases of 30, 15, and 5 minutes yielded values of 0.0519, 0.0495 and 0.0307 respectively. The first group reached a maximum ratio of 0.0894 on the seventieth day of the experiment and then rapidly declined. The 30-minute group reached a maximum of 0.0633 at the same time and showed less decrease. The 15-minute group reached a maximum of 0.0538 at a similar time but thereafter the rate remained constant for 32 days while the 5-minute group did not reach its maximum of 0.0325 until the ninety-second day. Controls exposed to natural daylight showed a rate which was still rising at 102 days. It is concluded that the rate of increase of light is directly related to rate of egg production.

57. REACTIONS OF TADPOLES TO ACIDS AND ALKALIS. H. T. Folger, Fisk University. (Lantern; 15 min.)

Folger (1939) has shown that hydrochloric acid makes tadpoles less positive or negative to light, and that ammonium hydroxide makes them more positive. Under the condition of these experiments, in which the acid or alkali was added to alkaline tap water, sodium hydroxide had little final effect, though, as, in the case of ammonia, its immediate effect was to make the tadpoles negative. Further investigation has confirmed these results, and added to them. Apparently all acids in appropriate concentrations make tadpoles negative, the degree of negativity depending upon the acidity. However, there appears to be some difference in the immediate effect of organic as compared with inorganic acids. Whereas mineral acids quickly cause tadpoles to become less positive, acetic acid, for example, may at first cause them to become more positive, an effect which is soon dissipated. The final effect of all acids appears to be the same. Tadpoles which have been made negative with an acid, can be made positive again with ammonium or sodium hydroxides

58. THE EXPERIMENTAL PRODUCTION OF MELANIN PIGMENT ON THE VENTRAL SURFACE OF THE SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*). Clinton M. Osborn, Ohio State University. (Lantern; 10 min.)

A technique of subjecting the ventral surfaces of adult summer flounders to continuous diffuse light of constant intensity will be described. Both direct and reflected light have been used with success. Preliminary experiments on 22 animals indicate that illumination of the normally unpigmented ventral surface causes pigment (in apparently normal melanophores) to develop there. The first macroscopic appearance of ventral pigment followed 7 days of continuous illumination at the highest intensity used. Considerable melanin can be grown in 30 days, and extensive pigmentation occurred in a few fishes treated 7 weeks.

All the flounders used in these experiments were in a physiological condition causing pigment dispersion in the melanophores on the dorsal surface. This was accomplished either by allowing these fishes to background-adapt to the black walls and ceiling of the experimental tank or by blinding fishes which previously had been black-adapted. Vision is not essential, therefore, to the experimental development of ventral pigment.

Fishes appeared to experience discomfort from ventral illumination as manifested in repeated attempts to avoid it by coming to rest on their edges with the ventral surface against a vertical wall.

59. ACTIVITY OF MELANOPHORES IN RESPONSE TO INJECTION OF ADRENALIN. Madeleine E. Pierce, Vassar College. (Lantern; 15 min.)

Injections of adrenalin regularly produce contraction of melanophores in cold-blooded vertebrates; and in *Fundulus*, at least, it has been proved that adrenalin works directly on the melanophores. The following experiments were undertaken to determine if any relation existed between the body weight of the animal and the period of time over which the melanophores remained contracted in response to the injection.

Normal time for blanching and darkening in response to background was determined for a series of animals varying sufficiently in weight. A constant volume (0.05 cc.) and known concentration of andrenalin was then injected. Time for blanching and darkening was again determined. Results from experiments with *Fundulus heteroclitus*, *Mollienesia latipinna*, and *Phrynosoma modestum* make possible the following generalizations:

1. Reaction time for contraction of melanophores is reduced; by 5-30 seconds in fish; by 1-1.5 hours in reptiles.
2. Reaction time remains constant within each species for all effective concentrations.
3. Positive correlation between concentration of adrenalin and duration of contraction of melanophores exists; the stronger the concentration, the longer the animal remains pale.
4. Positive correlation between the body weight and the duration of contraction of melanophores exists; the smaller the animal, the longer it remains pale. This correlation occurs at any concentration of adrenalin which is effective.

In experiments with *Phrynosoma douglassii* it was found that only the first three generalizations hold.

60. CONTRAST AND MELANOPHORE RESPONSE IN FISHES. R. N. Danielson, University of Minnesota and Oklahoma Agricultural and Mechanical College. (Introduced by D. E. Minnich.) (15 min.)

Various partly expanded states of the melanophores of *Nocomis biguttatus* and *Semotilus atromaculatus* result over a well illuminated white bottom when the light reaching these minnows from above is either relatively weak or completely excluded. The fish in situations where most of the light comes from below appear to adjust their shade in accordance with the degree of contrast in the visual field in the same way, though not to the same extent, as they do over comparable normally illuminated dark bottoms, where most of the light comes from above.

61. THE ACTIVE AND THE RESTING STATES OF MELANOPHORES TESTED EXPERIMENTALLY. G. H. Parker, Harvard University. (Lantern; 15 min.)

Fish melanophores will maintain a state of dispersed pigment, or of concentrated pigment, or of pigment in some intermediate position over long periods of time when they are kept in an unstimulating environment. The active states are not conditions of full dispersion or of full contraction as claimed heretofore, but are conditions of pigment movement as contrasted with conditions of pigment quiescence which are true states of rest. In this respect melanophores resemble smooth muscle-fibers and are unlike cross-striped muscle-fibers.

62. THE NEUROHUMORAL HYPOTHESIS AND THE COLOR CHANGES IN THE GOLDFISH, *CARASSIUS AURATUS*. Sears Crowell, Miami University. (Lantern; 15 min.)

Comparisons are made between the melanophore responses of innervated and denervated regions. The dispersion of pigment takes place in a region lacking nerves. However, this response is slower than that of innervated regions, and the melanophores do not attain maximal dispersion. Therefore nerves are believed to be necessary for full and rapid dispersion but neurohumors or hormones are also effective. Since a denervated region darkens uniformly rather than from the edges inward it is probable that a hormone rather than a neurohumor is the effective agent.

The concentration of pigment is as complete in a denervated region as in an innervated one. It is not, however, so rapid. The concentration in a region lacking nerves takes place uniformly rather than from the edges inward. In addition to nerves a hormone is believed to be effective in causing pigment concentration.

In its failure to show evidence of neurohumoral agents this animal differs from most of the fish which have been studied by other observers.

63. HUMORAL EFFECTS OF THE EYES IN AMPHIBIAN PIGMENT RESPONSES. Stephen W. Gray and Wanda M. Little. (Introduced by F. R. Steggerda.) Department of Physiology, University of Illinois. (Lantern; 6 min.)

Previous workers have assumed that the pituitary control of the frog melanophore system was under nervous control from the eyes. Blinded frogs become dark and lose their light background response.

In our experiments, eyes from light-adapted frogs were extracted in Ringer's solution and injected into both dark-adapted and blinded frogs. In both cases, contraction of the melanophores resulted. When dark-adapted eyes were injected into light frogs, expansion took place, although less consistently. The presence of intermedin or pars tuberalis hormone in the residual blood of the excised eye does not seem sufficient to account for these changes, as larger amounts of muscels from the same animal produced no effects. That the effect is through the pituitary, and not directly upon the melanophores, is evident from the fact that no change is produced in completely hypophysectomized animals.

There is also some evidence that extracts of the central nervous system of the frog have a greater melanophore effect than is to be explained by pituitary hormones in the residual blood.

64. COLOR CHANGES IN BLINDFOLDED AMERICAN CHAMELEONS, *ANOLIS CAROLINENSIS* (CUVIER). Francis H. Wilson, Tulane University. (Lantern; 10 min.)

In blindfolded American chameleons generally, darkness induces the green phase, but as intensity of illumination is increased the animal becomes a yellowish-brown with deeper browns resulting from still greater increases in light intensity. Local browning may be induced by exposing parts of the body to illumination of the intensity which just induces browning. Extreme heat 44°-50°C. induces greening even when exposed to light but below approximately 13°C. the lizard is brown whether in darkness or in the light.

65. THE MECHANISM CONTROLLING 24-HOUR CYCLES IN RETINAL PIGMENT MIGRATION IN THE CRAYFISH. John H. Welsh, Harvard University. (Lantern; 15 min.)

Crayfish (*Cambarus bartoni*) kept in darkness, at a constant temperature and fed at irregular intervals continue to show a diurnal rhythm in retinal pigment movements. These movements have been observed to persist for periods of 4 to 5 months. A hormone-like substance, probably from the sinus glands of the eye stalks, has been found to activate the retinal pigment cells. Since the sinus glands are under nervous control it is now possible to formulate a working hypothesis to account for the periodic release of the retinal pigment activating substance.

Illumination, the injection of extracts of eye stalks, and an internal change occurring during the day, when animals are kept in constant darkness, all result in the migration of retinal pigment to or toward the light position. Various investigators have also shown that low temperature, oxygen lack, anaesthesia, and death may result in the migration of crustacean retinal pigment to the light position. Therefore, it is suggested that the fibers innervating the sinus gland, and doubtless responsible for controlling the release of the retinal pigment hormone, are inhibitory fibers. Light, and factors which tend to lower metabolism, lower the activity of these inhibitory nerves allowing the release of the hormone. Such a concept of the inhibition of inhibitory impulses has previously been invoked to explain certain color change phenomena in reptiles, the release of the melanophore hormone from the pituitary of the frog, the control of bile secretion, and vasoconstriction by the depressor-vasoconstrictor reflex.

66. A COMPARATIVE STUDY OF THE ELECTRICAL RESPONSES FROM THE COMPOUND EYE. Frederick Crescitielli and Theodore L. Jahn, State University of Iowa. (Lantern; 15 min.)

Using 25 species of arthropods a comparative study has been made of the electrical responses obtained by illumination of the compound eye. In the crayfish, box elder bug, cricket, honey bee, king crab, and several species of butterflies, grasshoppers, and beetles the response of the dark-adapted eye to a brief exposure of bright light consists of a rapidly developing phase of electrical potential and a slow declining phase. The electrical polarity indicates a condition of electrical negativity of the illuminated eye with respect to the non-illuminated eye. The effect of light-adaptation varies with the species but in general the response is reduced and two distinct negative waves, a fast b-wave and a slower c-wave appear. In the Dobson fly and certain beetles the response of the dark-adapted eye also possesses an initial rapid positive element, the a-wave. In grasshoppers and butterflies an additional element, the d-wave, appears when the light is turned off. This is especially marked after a long exposure to a bright light. In a number of moths and beetles the response is characterized by the presence of a very prominent and long c-wave as well as a- and b-waves, and light-adaptation has an especially marked influence on the magnitude and form of the response. The effect on the electrical response of varying the duration of light exposure, the light intensity, the wave length, and the temperature has been investigated in many of the animals.

67. A DIURNAL RHYTHM IN THE ELECTRICAL RESPONSES FROM THE COMPOUND EYE OF CERTAIN BEETLES. Theodore L. Jahn and Frederick Crescitelli, State University of Iowa. (Lantern; 15 min.)

The electrical responses from the compound eyes of certain beetles follow a definite diurnal cycle. When these beetles are kept in total darkness and under approximately constant environmental conditions the electrical response to a brief exposure of bright light obtained during the morning and afternoon hours (day-type) is markedly different from the response obtained during the late afternoon and evening hours (night-type). The day-type of response is relatively simple and similar in many respects to the electrograms which have been recorded from the eyes of certain grasshoppers and butterflies. The night-type of response, always much larger, contains complex elements such as are seen in connection with the eyes of the *Cecropia* moth and other moths. This diurnal periodicity of the electrical potentials, which has been found to occur in *Chlaenius diffinis*, *Chlaenius tomentosus*, *Hydrus triangularis*, *Harpalus caliginosus*, and *Harpalus pennsylvanicus*, persists as long as the animal remains alive, which is as long as 4 days in some experiments. Light-adaptation of the eye reduces the magnitude of both the day-type and the night-type of responses, but the wave form is altered only in the case of the night-type of electrogram.

The possibility is discussed that this rhythm in electrical potentials is related to a diurnal migration of some of the eye pigments. Such diurnal variations in the movements of eye pigments have been observed in the case of a number of invertebrates.

68. FUNCTIONS OF THE TWO TYPES OF EYE IN THE BRINE SHRIMP, *ARTEMIA GRACILIS* VERRILL. John H. Lochhead, Harvard University. (Introduced by A. C. Redfield.) (Lantern; 7 min.)

The brine shrimp is provided with a median 'nauplius' eye and a pair of stalked compound eyes. Effective techniques have been devised for the removal of both these types of eye, without apparent damage to the health of the animals. Both normal and operated animals were tested for five different reactions to light: 1) phototaxis (antero-posterior orientation); 2) orientation of the ventral surface toward the light (dorso-ventral orientation); 3) turning at the boundary between light and dark regions; 4) temporary changes in swimming speed, following rapid alterations of light intensity; 5) convulsive contractions in a sudden bright light after a long period of darkness.

It was found that all these reactions are given by normal animals and by animals possessing only the two compound eyes or only one compound eye. Animals with only the nauplius eye give all except reaction (5), but their orientation is less precise, especially in the control of rotations round the transverse axis. Tests with very dim light passed through a Corning signal red filter showed that orientation still continues if either type of eye is present. All reactions to light disappear if both types of eye are destroyed, or if damage is done to the anterior transverse commissure of the brain.

The compound eyes are found to assist the males in their search for females, whereas the nauplius eye cannot be used in this way.

69. MECHANICAL FACTORS CONTROLLING SWIMMING POSITION IN AQUATIC INVERTEBRATES AND PARTICULARLY IN THE BRINE SHRIMP, *ARTEMIA GRACILIS* VERRILL. John H. Lochhead, Harvard University. (Introduced by A. C. Redfield.) (Lantern; 8 min.)

Mechanical factors may have an important influence on the body position of free-swimming invertebrates, more especially when statocysts are absent and when there is no stimulus by light. In many cases there will be a tendency for the center of gravity to lie below a center of support, the position of which will depend on the distribution of upward forces opposed to the downward pull of gravity. The upward force concerned may be buoyancy, or a direct propulsive force, or a lifting force such as that which supports an aeroplane in flight. Some mechanical orientation may also be provided by external pressure forces, similar to those which determine the position in which a coin will sink in water. The pattern of these forces will depend not only on the shape of the animal, but also on the currents which it creates.

In experiments with the brine shrimp it was found that blinded individuals usually swim in all possible positions, changing from one to another with great facility. If, however, the animals are lethargic, they swim lying on the back, doing this whether the brine is of a specific gravity less than, equal to, or greater than that of the individual. The center of gravity was found to lie just slightly dorsal to the center of buoyancy, a fact which at least partially explains the experimental results obtained.

70. THE RESPIRATORY METABOLISM OF NORMAL AND GENETICALLY DEFICIENT EGGS OF *DROSOPHILIA MELANOGASTER*. E. J. Boell and D. F. Poulson, Yale University. (Lantern; 10 min.)

The use of the Cartesian diver ultra-micromanometer permits an analysis of the effects of genetic constitution on the respiratory metabolism of single *Drosophila* eggs.

The oxygen consumption of normal, wild-type eggs is uniform with time and shows a slight tendency to increase with age during the first 12 hours. The rate of oxygen uptake averages 0.026 cu. mm. per egg per hour, and so far no sex difference has been detected.

The respiratory rate of unfertilized eggs is, from the beginning, lower than that of normal eggs. The initial rate is approximately three times greater than the final value of 0.005 cu. mm. per hour.

In eggs deficient for the X-chromosome (YY) respiration, at the outset, is indistinguishable from that of normal eggs. Several hours after laying, the oxygen consumption declines to a final value one-fifth that of normal eggs. This is maintained at a steady rate similar to the equilibrium value of unfertilized eggs for at least 14 hours. The initiation of respiratory breakdown in No-X eggs coincides in time with developmental failure. Cytological examination of eggs which fail to develop was made in order to distinguish between unfertilized and deficient eggs.

Eggs which contain, in addition to the normal chromosome complement, an extra Y (XXY) or X (XXX) show the same rate of oxygen uptake characteristic of normal eggs.

The data indicate that while developmental failure is accompanied by a corresponding failure in respiration, the mechanism for oxygen uptake in deficient eggs is fully functional during the initial stages after fertilization.

71. RESPIRATORY METABOLISM OF MAMMALIAN EGGS AND EMBRYOS. E. J. Boell and J. S. Nicholas, Yale University. (Lantern; 10 min.)

The respiratory metabolism of rat eggs and embryos during early development has been measured by means of the Cartesian diver ultra-micromanometer.

The technique employed in the handling of eggs and embryos for respiratory studies is similar to that used in experiments on isolated tissue slices, and affords essentially linear oxygen uptakes over a period of several hours.

The oxygen consumed by uncleaved and two-cell eggs averages approximately 0.0007 cu. mm. per egg per hour. During subsequent cleavage, leading to the production of 16 cell and morula stages, this figure is increased in embryonic embryonic growth occurs the oxygen consumed reflects the increase in embryonic mass rising from 0.01 cu. mm. per hour for an embryo on the eighth day after fertilization to one of 0.2 cu. mm. per hour on the tenth day.

The influence of various metabolites on the maintenance of respiration of embryos in vitro suggests that carbohydrate is a primary energy source during early mammalian development.

The Q_{O_2} (calculated on a dry weight basis) decreases steadily during development from a peak of approximately 30, for egg stages, to values of 12 to 15 for early somite embryos. Studies on isolated tissues indicate that the metabolic characteristics of adult tissues are differentiated early in embryonic development.

72. THE OXYGEN CONSUMPTION OF THE CHICK EMBRYO AT VARIOUS EARLY STAGES OF DEVELOPMENT. Fred S. Philips, University of Rochester. (Introduced by B. H. Willier.) (Lantern; 15 min.)

The oxygen consumption of whole unincubated blastoderms and embryos of primitive streak, head process, and early somite stages through the third day of incubation has been determined in an atmosphere of 100% O_2 in the Fenn micro-respirometer. Rates have been compared as c.mm. O_2 /hr./mg.N (designated as Q_{O_2}).

The Q_{O_2} of unincubated blastoderms increases in a linear fashion as temperature increases from 24 to 38°C.; at 38°C. the rate almost doubles between the second and seventh hour of measurement. The Q_{10} for this temperature range is about 3. These data do not reveal any striking changes in oxygen consumption as might be expected with the high incubation temperatures necessary for development.

The rate of oxygen consumption of the embryos of the primitive streak, head process, and early somite stages through 3 days of incubation decreases with time in a glucose-free medium. This decrease is reversible and is prevented by addition of glucose. For embryos through 2 days of incubation the rate is not limited by oxygen diffusion; it may be for the 3-day embryo. However, the oxygen consumption of 4- and 5-day embryos is limited by oxygen diffusion. Contrary to expectation the rate per unit of tissue declines insignificantly with advance in development from 15 to 72 hours of incubation. Furthermore, the respiratory rate of the early chick embryo is of the order of many adult mammalian tissues.

73. HYDROGEN-ION CONCENTRATION OF THE ALLANTOIC AND AMNIOTIC FLUIDS OF THE DEVELOPING CHICK. Paul A. Walker, University of Connecticut. (Lantern; 15 min.)

Measurements of the hydrogen-ion concentration of these fluids in individual chicks have been made using the glass electrode technique. The allantoic fluid shows a slight rise in pH between the 7th and 8th days (from 7.4 to 7.6) falling again to 7.4 by the 11th day. This value is maintained till the 13th day, when the pH diminishes rather rapidly to definitely acid values at the end of development. The maximum change occurs between the 14th and 16th days.

The amniotic fluid is slightly less basic (pH 7.1) at the start. On the 10th day, it has become very slightly acid (6.9). This decrease in pH continues till the 15th day, when a minimum of 6.4 is attained. From this time on, pH increases rapidly, reaching 7.9 on the 19th day.

The impermeability of the amnio-allantoic membrane to ions is very evident, particularly at the end of incubation. The change from basic to acid conditions in the case of the allantoic fluid may perhaps be correlated with the transition from mesonephric to metanephric excretion. The low pH values found on the 19th day (5.55) approach those of adult urine.

These results show a remarkable agreement with the data of Yamada ('33) who employed a different technique. The work of Aggazzotti ('13), which has long been accepted, is at variance with both these sets of data and is shown to be in error.

74. SIZE OF EMBRYONIC ORGANS AS DETECTOR OF ORGAN-SPECIFIC SEROLOGICAL EFFECTS. Paul Weiss, University of Chicago. (Lantern; 5 min.)

Guinea pigs were injected in three groups with extracts of chicken liver, kidney, and muscle, respectively (collaborator: D. H. Campbell). Antisera recovered from the treated animals were injected into chick embryos of 1½, 3½, 5 and 8 days' incubation. In 108 embryos (experimental and control) killed at 19 days incubation age the weights of liver and kidney (metanephros) were determined and evaluated statistically. Significant differential effects of the organ-specific antisera on the size of the test organs were noted. Embryonic growth can thus be used as detector of biochemical organ differentials.

75. SKIN TRANSPLANTATION IN ADULT RANA PIPIENS: AUTOGRAFTS COMPARED WITH HOMOIOGRAFTS. Howard H. Vogel, Harvard University. (Introduced by Herbert W. Rand.) (Lantern; 15 min.)

A new type of skin transplant, composed of an anterior autograft and a posterior homoiograft, both placed within the same wound area, was used to study the problem of incompatibility of homoiotransplants.

Autografts usually showed a good circulation within 1 week after transplantation. In both pigmented and unpigmented autografts, specificity was retained. No pigment migrated into or out of an autograft of frog skin. Some autografts were observed for a period of 6 or 7 months.

Homoiografts showed a characteristic capillary dilation shortly after transplantation. This 'vascularity reaction' was studied carefully. Unpigmented trans-

plants were invariably invaded by host cells shortly after transplantation. The invasion by host epidermis, and by lymphocytes and leucocytes was studied in all homoiografts. Also the regeneration of glands from the new epidermis was observed. Circulation was rarely seen in homoio-transplants until after the host epidermis had replaced that of the graft. This lack of circulation seemed closely associated with the incompatibility of the homoiografts.

Autotransplants were placed within the surrounding areas of homoio-transplants. The reciprocal exchange of grafts was also attempted.

Homoiografts were destroyed equally well on splenectomized and hypophysectomized frogs. The reticulo-endothelial system was blockaded with vital dyes, but this did not prevent the lymphocytic invasion of the homoiograft. No protection was afforded the transplants by grafting skin that had first been implanted into the lymph sac of the host, nor did injections of solutions of the donor's skin into the host's lymph sac prior to transplantation prevent the invasion and eventual destruction of the homoiografts.

76. OBSERVATIONS ON SPECIES DIFFERENTIALS IN BIRDS AS STUDIED BY MEANS OF HETERO-TRANSPLANTATION OF EMBRYONIC LIMB BUDS. Herbert L. Eastlick, University of Missouri and the University of Chicago¹. (Lantern; 15 min.)

Limb buds of duck, chukar partridge, quail and guinea fowl embryos have been transplanted to the body wall of 60-70 hour chick embryos. Forty-eight hosts have hatched. In varying periods of time after hatching all transplants present evidence of host-graft incompatibility but the time of its appearance as well as its severity varies in the different species.

A duck graft grows and appears normal up to 10-14 days after hatching at which time swelling of the web and toes occurs followed by an edema in the entire leg, rupture of the skin in a number of places, extravasation of plasma and loss of feathers. The healing process extends over a period of several weeks beginning at the proximal portion of the graft and extending distally. During this time the graft continues to grow. Chick skin apparently replaces that of duck, and feathers, seemingly of host origin, develop upon the graft. The syndrome in chukar partridge and quail transplants is similar but such grafts are unable to persist and are resorbed, the latter more rapidly than the former. Guinea fowl grafts appear to be the least compatible since they grow very little after hatching. The foot and toes usually blacken in 2-6 days and the entire graft degenerates rapidly.

Although the factors underlying the above reactions have not been investigated, the time at which the syndrome is initiated suggests the possibility that an immunity to the graft has been developed by the host.

¹ National Research Council Fellow.

77. ALTERATION AND REGENERATION IN THE HEAD AND PHARYNGEAL REGIONS OF PLANARIANS. Frank M. Hull, University of Mississippi. (Lantern; 15 min.)

This paper records first the results of cuts and the removal of tissue from the head region of planarians in order to compare the effect upon the organism with the result of earlier studies involving the pharyngeal region. Secondly it records

preliminary experiments upon the behaviour of segments taken from the central region of a planarian. The species used was *Dugesia tigrina* Girard. Short cuts just behind the head region, if kept open appear to mechanically isolate a part of a planarian from the dominance of the head region and resulted in the formation of a well developed new head, usually extended more or less vertically from the dorsal surface of the base-individual. The removal of segments and blocks of tissue result usually in a hollow 'chimney-headed' planarian developed upon the upper surface of the base-individual.

Large oval segments taken from the pharyngeal region of the planarian remain alive and relatively or totally inactive for considerable periods of time during which there is neither regeneration nor disintegration. In those instances studied disintegration has finally resulted but may have been due to unfavorable circumstances.

78. POLYDACTYL FEET IN TWO STRAINS OF CHICKS. Mary T. Harman and Frances Nelson, Kansas State College. (Lantern; 5 min.)

The nature of polydactylism was studied in 9 to 12 day embryos and newly hatched chicks. The feet were prepared by the alizarin method.

The polydactylism was manifested by the duplication of the hallux (duplicate polydactyl), and the increase in the number of phalanges (polyphalangeal polydactyl). The parents of the duplicate polydactyl chicks were, for the most part, from the F_1 generation of normal by duplicate polydactyls. Three hundred thirty-seven of this type were studied.

Of these, 22 feet were normal, 166 had two halluces, 105 had three halluces, four had four halluces, five had a single long hallux, and 35 were quite irregular.

The polyphalangeal polydactyl strain was from matings of selected individuals showing this type, and from the regular strain of polydactyl chicks. The hallux of this strain was long and most often consisted of four or five phalanges instead of the normal number, three. Of the 132 feet of this strain examined, there were 85 with one long hallux, 22 with one long hallux and an extra bone, 8 with one long and one short hallux, and 17 with two long halluces.

The shapes of the metatarsals in both classes ranged from the normal to a large club-shaped bone. Many specimens had fewer metatarsals than halluces.

Eighty-seven pairs of the duplicate polydactyl feet were similar, while 72 pairs had bilateral variation. In the polyphalangeal polydactyl strain 23 pairs were similar and 43 pairs had bilateral variation.

79. DEVELOPMENT OF THE EXTRA-EMBRYONIC CIRCULATORY SYSTEM AND THE BLOOD PICTURE OF CHICKS INCUBATED UNDER INCREASED ATMOSPHERIC PRESSURE. Bert Cunningham and L. J. Flemister. Duke University. (Lantern; 10 min.)

Hen eggs incubated under increased atmospheric pressure show a retarded development of the extra-embryonic vessels and in addition the blood picture shows an earlier embryonic condition that is to be expected for normally incubated embryos of the same age.

80. INFLUENCE OF TEMPERATURE ON THE RATE OF EARLY EMBRYONIC DEVELOPMENT. Alexis L. Romanoff, Department of Poultry Husbandry, Cornell University. (Lantern; 15 min.)

The observations are made on twenty-three groups of eggs of *Gallus domesticus*, similar in genetic and environmental history, incubated at various temperatures ranging from 23.5° to 45.5°C. At intervals of 12 hours a number of eggs were removed from each group for whole mounts of the embryo.

The measurements made from these mounted embryos reveal that within the range of possible hatch, that is, between 35.25° and 40.25°C. [cf. Poultry Sci. 15: 311 (1936); Cornell Univ. Agr. Exp. Sta. Memoir 216: 1 (1938)], the increase in the rate of total growth with the increase of temperature can be expressed by the straight line relationship. The response of the embryo within this thermal region is therefore poikilothermic. Below 35.25°C. or above 40.25°C. the growth rate was invariably retarded and abruptly affected in a special manner. The increasing incidence of various degenerative processes in the development indicates the pathologic effects of these 'abnormal' temperatures.

81. GENETIC ASPECTS OF PIGMENT PRODUCTION IN THE GUINEA PIG. Mary T. Harman and Annette Alsop, Kansas State College. (Lantern; 10 min.)

The earliest stages of pigment formation in the hair and skin of guinea pigs are seen in fetuses of 43 days when all pigment granules look alike. In older fetuses and in adult animals, black granules are present in both the medula and cortex of black hairs, chocolate granules in chocolate, and red granules in red hairs.

No black or chocolate granules are seen in red hairs. Possibly the factors B and b have some effect on the shade of the red granules in red hairs. No colored granules are seen in c^r or c^a white hairs. Colorless granules are present in these hairs in a position corresponding to that of pigment granules in pigmented hairs.

Black and chocolate pigment granules are present in the skin of B- and bb animals. No red granules are seen in the skin.

Diffuse reddish pigment is seen only in the cortex of the fully formed hair in association with black, chocolate, or red granular pigment. Hence, it is seen in C- red, chocolate, and black hairs, and in c^r chocolate and black hairs, but not in c^r and c^a white hairs. Treatment with 15% sodium hydroxide solution indicates the possible presence of a dilute form of red diffuse pigment in white hairs.

Preliminary work on the histological and cytological origin of pigment in the guinea pig indicates that the pigment cells are ectodermal in origin and that pigment granules are not formed directly from the nucleus of the pigment cell.

82. POLYEMBRYONY AND DELAYED IMPLANTATION; THEIR OCCURRENCE AMONG THE GENERA OF ARMADILLOS. G. W. D. Hamlett. (Lantern; 10 min.)

Since 1909 it has been known that armadillos of the genus *Dasypus* reproduce polyembryonically; moreover, they are the only vertebrates in which this one-egg twinning is known to be the habitual mode of reproduction of an entire species. We have hoped that a study of other armadillo genera might reveal more primitive stages in the evolution of this extremely specialized embryological process.

This hope must be abandoned. During a recent stay in South America on a Guggenheim Fellowship, I have accumulated enough evidence to indicate that it is the species of *Dasypus* alone which show any trace of polyembryony. The other genera (possibly excepting *Chlamydophorus*, for which data are lacking) give birth to single young or to two-egg twins. Of the five subfamilies, only the *Dasypodinae* seem to have discovered the secret of making four embryos grow where only one egg is implanted.

Accompanying its polyembryony, *Dasypus* possesses the curious habit of delaying the implantation of the blastocyst until several months after fertilization. This has apparently no relation to the polyembryony, as delayed implantation is found in many other mammals which are not polyembryonic; nevertheless it is interesting that apparently only *Dasypus* among the armadillos shows this peculiar behavior of the implantation mechanism.

83. THE EFFECT OF PRIMING DOSE OF OESTRADIOL AND LENGTH OF THE PRETRAUMA PERIOD ON PLACENTOMATA SIZE. Irving Rothchild and Roland K. Meyer, University of Wisconsin. (Lantern; 10 min.)

Forty adult female rats, castrated for 2 months, were divided into 6 groups, and given 0.05, 0.10, 0.22, 1.3, 6.7 and 33 gamma of oestradiol respectively, over a 24 hour period. Fifteen long-time castrates were given no oestrogen, while a group of 12 normal adults were castrated in heat. Progesterone was injected for 8 days after the oestrogen treatment, with trauma of the uterus on the fourth day, and autopsy on the ninth. The dose was 0.6 Rb.U./day/rat in all animals except 6 of the 15 given no oestrogen treatment, where it was 2 Rb.U./day/rat.

All rats given oestrogen (including those castrated in heat) formed placentomata. No significant relation could be demonstrated between oestrogen dose and placentomata size. No placentomata formed in those rats receiving 0.6 Rb.U./day without previous oestrogen treatment, but did in the rats which received 2 Rb.U./day.

Fifty-five adult female rats, castrated in heat, were injected with 0.6 Rb.U. of progesterone/day for 2, 4, 6, 8, 10 and 12 days before the uterus was traumatized, and for 5 days afterward. All animals except those traumatized 2 days after heat formed placentomata. The maximum size occurred in those rats traumatized 4 days after heat, the minimum, in those rats traumatized 12 days after heat.

84. EFFECTS OF PROSPERMIN ON OVARIES AND UTERI OF ANESTROUS GROUND SQUIRRELS. L. J. Wells, University of Missouri. (Lantern; 15 min.)

Thirty immature and 22 anestrous female ground squirrels (*Citellus tridecemlineatus*, a monestrous rodent) were used in this study. The right ovary and uterine horn were removed surgically from adults, weighed, sectioned and stained. Ovaries contained several follicles with small antra. Uteri were essentially infantile in character. Prospermin (Squibb), an extract of human male urine, was administered to 24 immature and 12 adult females. Subcutaneous injections of 0.5 to 10.0 milligrams of prospermin in distilled water were made twice daily for 10 days. Injected females and uninjected controls were killed on the eleventh day. Remaining ovaries and uterine horns were removed and weighed. Ten-micra serial sections of all ovaries and 6 μ sections from all uterine horns were stained.

All prospermin recipients showed rupture of the vaginal membrane on the seventh to tenth day, and growth of ovaries and uteri. Weight of ovaries and uteri from treated animals was respectively, 2.9 to 22.6 and 5.9 to 18.2 times greater than that for those from control females.

Ovaries of injected animals contained large follicles, follicles with blood in their cavities and corpora lutea. Ova were observed in bloody follicles and corpora lutea. Ovulation probably did not occur.

Growth of the endometrium and myometrium to levels characteristic of proestrus or estrus occurred in each injected female. Patches of metaplastic stratified epithelium, resembling those induced by large doses of estrone, were observed bordering the uterine lumen in 10 of the 24 injected immature animals.

85. INDUCTION OF OVULATION IN THE BAT WITH THE PREGNANCY URINE FACTOR.

Mary J. Guthrie and Elizabeth W. Smith, University of Missouri. (Lantern; 10 min.)

Ovulation was induced in 13 out of 18 (72%) mature *Myotis grisescens* females by injection of Antuitrin-S in November, and in 37 out of 40 (92%) in December. When Thyroxyl (Squibb) was administered with Antuitrin-S in November ovulation occurred in every specimen (13). Ovulation was not induced when adrenal cortex extract (Upjohn) was combined with Antuitrin-S (10 individuals), and the addition of cortical extract to the Antuitrin-S and thyroxin dose reduced the incidence of ovulation to 6 out of 9 cases (66%).

Since follicles with antra appear in the ovaries of *M. grisescens* in August attempts were made to stimulate ovulation with Antuitrin-S beginning August 24, but without success in 19 individuals. On September 9, when the bats had stored much fat in advance of hibernation, results were again negative in 16 cases, although the dose was doubled. Insemination had occurred in 3 out of 8 bats which had not migrated on September 26. Three of these females ovulated after the same dosage with the same sample of Antuitrin-S that was used earlier in the month, but there was not complete coincidence between insemination and ovulation. Of 20 specimens obtained on October 7 after migration all were inseminated, but only 4 were induced to ovulate. It would appear that the bats tested react to this gonadotropic factor only after the onset of estrus as measured by receptivity to males, but it is not clear why sensitivity rises so slowly.

86. THE EXPERIMENTAL SHORTENING OF PROLONGED GESTATION IN THE LACTATING ALBINO RAT. Charles K. Weichert, University of Cincinnati. (Lantern; 15 min.)

Lactating rats inseminated within 24 hours after parturition have gestation periods ranging from 22-36 days. The prolongation is closely correlated with the number of suckling young. Other workers have demonstrated that a similar phenomenon occurs in mice and is due to delayed implantation, but the factors involved have not been explained.

That the delay in implantation is due to some endocrine imbalance has been demonstrated. Inseminated lactating rats suckling 8-11 young were injected with varying amounts of Antuitrin-S. Best results were obtained in animals injected daily for the first 8 days after insemination with 10 ru. of Antuitrin-S. The animals were sacrificed on the 21st day of gestation. Fully developed, normal

living fetuses were found. The number of fetuses was small and resorption areas were present.

Another series injected with synthetic progesterone did not show a comparable hastening of implantation and subsequent development. However, if a series of Antuitrin-S injections was supplemented with administration of progesterone, the uterus with its contained fetuses was indistinguishable from a normal uterus the day before parturition even though as many as 10 young were being suckled.

It is suggested that the act of suckling may stimulate the production of prolactin by the hypophysis with a corresponding inhibition of secretion or release of gonadotropic hormones. The time when ovarian hormones necessary for nidation are released may thus vary with the strength of the suckling stimulus and therefore be dependent upon the number of suckling young.

87. EFFECTS OF ADRENALECTOMY ON VAGINAL AND UTERINE RESPONSES TO ESTROGENS. Robert L. Kroc, Indiana University. (Lantern; 15 min.)

Successive assays of theelol were made on ovariectomized young adult rats before and after adrenalectomy. The percentage exhibiting cornified smears was greatly increased following adrenalectomy and increased still more when the assays were continued on those still living but showing marked weight losses. Thus, the vaginal sensitivity to estrogen was definitely increased by adrenal removal.

The uterine weight response to estrogens was determined on immature rats by the Astwood 6-hour test method. Whereas adrenalectomy 1 day prior to the test reduced the uterine response to a given dosage of theelin, adrenalectomy 3 days prior to the test was followed by the opposite result. Adrenalectomy alone or sham operation without estrogen injections reduced the uterine weights whether the operations were performed 1 to 3 days prior to autopsy. Pinching of the adrenal connections alone resulted in marked increases in uterine weights. Estrogen plus pinching resulted in uterine weights greater than those obtained from unoperated rats treated with the same dosage. The relations of these results to the physiology of the adrenals and ovaries will be discussed.

88. FUNCTION OF THE LUTEAL-LIKE CELLS IN THE MOUSE OVARY. Carroll A. Pfeiffer and Charles W. Hooker, Yale University. (Lantern; 15 min.)

In mice of the Strong A strain which have received pregnant mare serum (20 RU daily) for 60 days the ovaries are composed almost entirely of masses of luteal or luteal-like cells. Some of these masses are corpora lutea, while others are evidently luteinized primary follicles, transformed cords of cells from the germinal epithelium, or even transformed interstitial cells. The uteri of these animals showed a response which could conceivably have been elicited by the action of estrogen plus either androgen or progestin. To ascertain which, if either, hormone has augmented the estrogen and with a view to determining the function of the luteal-like cells, castrated males of the same strain bearing ovarian ear grafts have been given the same treatment. The grafted ovary responded in the same manner as the ovary in situ and the seminal vesicles showed definite signs of androgen stimulation within 35 days and restoration to complete normality in 60 days. Transplants of the stimulated ovaries and of the stimulated grafts quickly fibrosed and at no time could androgenic action be detected despite continuation

of mare serum. Castrated males without grafts receiving mare serum and uninjected hosts showed no androgen effects upon the seminal vesicles in a period of 3 months. Sixty-five to eighty-five daily injections of 20 RU of PU, 2 mg. of AP, 2 mg. of LH, and 2.5 RU of FSH each resulted in estrogenic stimulation of the seminal vesicles of the grafted animals, with PU the most effective in this respect.

89. THE TIME AND SEQUENCE OF PREOVULATORY CHANGES IN THE OVARY OF THE CAT FOLLOWING MATING OR MECHANICAL STIMULATION OF THE CERVIX. Alden B. Dawson, Biological Laboratories, Harvard University and Harry B. Friedgood¹, Department of Physiology, Harvard Medical School. (Lantern; 15 min.)

Ovulation in the cat according to most data usually occurs 24 to 30 hours after mating or a comparable stimulus. During the first 6 hours there are no obvious morphological changes although the follicles increase in size due to the continued secretion of primary follicular fluid. After this period changes occur which are associated with the production of the secondary fluid. The reaction is first seen in the cumulus and gradually involves the entire granulosa. The cumulus enlarges; the cells become widely separated due to intercellular accumulations of secretion and the corona gradually becomes more prominent. Between 10 and 12 hours the membrane of the germinal vesicle disappears and chromosomes emerge. At 14 hours dense tetrads are seen and at 18 hours the spindle of the first maturation division is present. At 22½ hours a first polar body is given off and the spindle of the second division is formed. No further maturation changes occur in the ovum until after fertilization. The entire granulosa participates in the production of secondary secretion which first accumulates in intercellular spaces around which the follicular cells are arranged in rosettes. Later the cells become irregularly distributed and the interstitial secretion streams into the antrum. Secondary and primary secretion can usually be distinguished histologically and the interface between them frequently appears membranous. By 24 hours the production of secondary secretion is virtually over. Previous to ovulation the follicular cells are enlarged, suggesting an early stage of transformation into luteal cells. Their nuclei lose their pyknotic appearance and become vesicular.

¹ Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

90. THE NORMAL REPRODUCTIVE CYCLE OF THE FEMALE RAT AS DETERMINED BY MEANS OF THE COPULATORY RESPONSE. Richard J. Blandau, John L. Boling and William C. Young, Brown University and Yale University. (Lantern; 8 min.)

Normal reproductive cycles of 114 adult female rats (Wistar strain) have been determined by the use of the copulatory response, elicited in a manner similar to that described for the guinea pig by Young, Myers and Dempsey. The onset of 687 heat periods was distributed throughout the day as follows: 17% from 11 A.M. to 5 P.M.; 72% from 5 P.M. to 11 P.M.; 11% from 11 P.M. to 5 A.M.; and 0.5% from 5 A.M. to 11 A.M. The average duration of 608 heat periods was 13.7 hours, with a range of 1 to 28 hours. The length of heat was not significantly altered in 14 animals allowed to copulate at the beginning of heat. Sixteen periods of heat corresponding to the 'split oestrus' observed in the guinea pig were re-

corded. The length of the cycle was determined in 567 cases with the following distribution: 2 days, 1%; 3 days, 5%; 4 days, 58%; 5 days, 24%; 6 days, 5%; 7 days, 2%. The remaining 5% of the cycles were between 8 and 18 days in length. The relationship between heat and the vaginal condition will be discussed.

91. THE TIME OF OVULATION IN THE FEMALE RAT IN RELATION TO THE BEGINNING OF HEAT. John L. Boling, Richard J. Blandau, and William C. Young, Brown University and Yale University. (Lantern; 7 min.)

The time of ovulation in relation to the beginning of heat, as determined by the copulatory response, has been investigated in 46 female rats (Wistar strain). When the onset of heat had been established, the ovaries and oviducts of all animals were examined at given intervals after the beginning of heat for the presence of ruptured follicles and ova. The results were as follows: 2 of 12 animals had ovulated by $7\frac{1}{2}$ to $8\frac{1}{2}$ hours after the beginning of heat; 6 of 11 by $8\frac{1}{2}$ to $9\frac{1}{2}$ hours; 8 of 8 by $9\frac{1}{2}$ to $10\frac{1}{2}$ hours; 4 of 6 by $10\frac{1}{2}$ to $11\frac{1}{2}$ hours; and 11 of 12 by $11\frac{1}{2}$ to $12\frac{1}{2}$ hours. Small numbers of eggs recovered in 6 of the animals which had ovulated indicated that ovulation was probably not complete at the time of examination. All ovaries are being sectioned for microscopical study to confirm the results of the original observation. Further observations are being made to determine the range of occurrence of ovulation on either side of the average time indicated by the above data.

A study of vaginal smears taken at the time of examination for ovulation indicates that ovulation usually occurs about the time cornification is complete; however, variations in this relationship occur.

Studies of ovulation involving a small number of animals of two other strains will be reported.

92. MUCUS-CONTAINING EPITHELIUM IN THE NORMAL GUINEA PIG UTERUS. Hugh I. Myers and Helen Rhoda Chornish, University of Richmond. (Lantern; 15 min.)

In the normal guinea pig the epithelium of the middle portion of the body of the uterus contains mucus at all times. However, the extent of the area involved changes during the reproductive cycle. From the first to the eighth day of the dioestrus the mucus area spreads both anteriorly toward the junction with the horns of the uterus and posteriorly toward the cervix and vagina. From then until the next oestrous period the upper boundary recedes toward the middle of the body of the uterus, while the posterior extremity continues its migration until at the prooestrus both the cervical and surrounding vaginal epithelium contains an abundance of mucus.

During the oestrous period the mucus is liberated in part in the body of the uterus, cervix and surrounding vaginal region by the dissolution or rupture of the epithelial cell surfaces. In addition, the cervical and surrounding vaginal regions exhibit a rapid epithelial growth with the formation of a cornified layer between the newly formed cells and the mucus-containing cells. Both cornified and mucus-containing cells are sloughed off practically intact. However, in the body of the uterus the epithelial cells slough off singly or in patches, being replaced as rapidly as removed by new epithelium which immediately elaborates mucus.

The relationship of these changes to the known cyclic changes in the ovary and hypophysis will be discussed.

93. EFFECTS OF GONADECTOMY ON THE INVOLUTED THYMUS OF ALBINO RATS. James C. Plagge, The University of Chicago. (Introduced by Carl R. Moore.) (Lantern; 8 min.)

One month after albino rats of more than 206 days of age were gonadectomized, their involuted thymus glands showed increase in weight of from 100% to 300% over normal. Eighty-two male and female rats, including test and control animals, were castrated between the ages of 206 and 546 days, during which period the thymus has involuted to less than 50% its maximum weight. The animals were sacrificed at intervals ranging from 10 to 70 days after operation.

In many animals the thymus returned to nearly its normal maximum weight of 250 to 300 mg., which in our colony is attained at about 80 days of age. Gonadectomy in 500-day-old rats, in some of which the thymus weighed only 50 mg., resulted in the same percentage of hypertrophy as observed in the less involuted glands of 250-day-old rats. In both gross and histological appearance the hypertrophied thymi resembled those of normal animals at 80 days of age.

Thymus weights were checked against unoperated littermate controls, sacrificed both on the day of operation and at autopsy of castrated animals.

In this series rats with severe lung infections showed little or no hypertrophy of the thymus after castration.

94. ADRENAL, THYROID, AND PITUITARY WEIGHTS OF EMOTIONAL AND NON-EMOTIONAL RATS. Eleanor H. Yeakel and Ruth Pinkney Rhoades, Western Reserve University. (Introduced by Mary E. Collett.) (Lantern; 10 min.)

This work represents the first step in an effort to determine any anatomical or physiological differences which might exist between emotional and non-emotional rats. The animals were bred and raised in the Psychology Laboratory of this University and their emotional ratings determined by Dr. Calvin S. Hall, who then kindly gave the animals to us.

Each rat was fasted for 17 hours or longer, killed with ether, and weighed. The adrenal, thyroid, and pituitary glands were removed and weighed rapidly, and the relative weights calculated. The figures were then grouped according to the sex, age, and emotionality of the animals. Two hundred and thirty-eight rats have been autopsied thus far.

The emotional rats have heavier body weights than non-emotional animals of the same age and sex. The relative weights of the glands show that the emotional animals also possess heavier adrenals, thyroids, and pituitaries. These differences, while not marked in all groups, are nevertheless consistent.

95. THE VACUOLE SYSTEM OF A FRESH WATER AMOEB. Dwight L. Hopkins, Mundein College. (Lantern; 15 min.)

The food vacuoles of this amoeba arise through the origin *de nova* from the protoplasm of small clear vacuoles which swell and coalesce with each other and with engulfed food. When the amoeba ceases feeding, small vacuoles containing refractile granules are formed instead of small clear ones. These granular vacuoles do not swell osmotically. The granules may be stained intra-vitally with neutral red, or Nile blue sulfate or Janus green B.

Thus the origin and activities of the food vacuoles in this fresh water amoeba are almost identical with those of the marine amoeba, *Flabellula mira*, which I have previously described.

Unlike *Flabellula mira*, this amoeba has a second vacuole system, the contractile vacuole system, which arises through the origin de nova from the protoplasm of other small clear vacuoles which swell and coalesce with each other. The contractile vacuoles may continue to swell, coalesce and empty to the outside after feeding and the growth of food vacuoles have ceased. Under certain unfavorable conditions, however, such as the presence of a relatively high concentration of Janus green B, the vacuoles of the contractile vacuole system become granular and cease to grow by swelling. These granules may be stained with Janus green B, but not with neutral red, or Nile blue sulfate.

The observations indicate that the contractile vacuoles as well as food vacuoles swell due to the solution of osmotically active substances in them and that swelling may be prevented by the isolation of this substance as an osmotically inactive precipitate or coagulum.

96. THE MEASUREMENT OF THE EFFECT OF REDUCED GLUTATHIONE UPON THE INITIATION OF MITOSIS IN AMOEBA PROTEUS SELECTED IN LATE INTERKINESIS. H. W. Chalkley, National Cancer Institute, Washington, D.C. (Lantern; 15 min.)

Selected groups of amoebae from standardized cultures yield approximately 25% fissions in a 3-hour period and 38% in a 4-hour period. Cumulative plotting of the initiation of fission shows that using 500 cells the rate of initiation of fission during the first hour is about 0.4 cells per minute, and thereafter about one cell per minute. The addition of M/5000 GSH raises the rate to 1.65 cells per minute, of M/10,000 GSH to two cells per minute and of M/15,000 GSH to 1.3 cells per minute, M/20,000 GSH shows no stimulation of rate during the experimental period. The effect is not immediate. There is a latent period which varies in an inverse ratio to the concentration of GSH. The addition of food to controls does not affect the rate. The low rate during the first hour is due to handling and change of fluid when the cells are transferred from culture to a standard medium for observation.

97. THE SIZE OF THE MOLECULES IN THE GROWTH SUBSTANCE PRODUCED BY CHILOMONAS PARAMECIUM. S. O. Mast and D. M. Pace, Johns Hopkins University. (15 min.)

Four 20 cc. sacs were made of cellophane membranes, fastened to glass tubes and suspended in flasks containing culture fluid. Culture fluid was put into each sac and chilomonads added to that in the sac in one flask (1), to that around the sac in another (2), to that in the sac and around it in still another (3), and to neither in the remaining (4) and left until they had died out in all. Then culture fluid was taken from flasks 1 and 4 and the time required for division of chilomonads in it measured.

The chilomonads lived 8 days in (1), 6 in (2), and only 4 in (3). It required 9.5 hours for division in fluid from 1 and only 6.5 in fluid from 4.

In other experiments in which fluid was taken from the flasks at intervals the time required for division in fluid taken from (1) increased from 5.66 hours in

fluid 15 hours old to 6.54 hours in that 96 hours old, but in the fluid taken from (4) it was 6.33 hours with no increase with age.

These results prove that the growth substance passed through the cellophane membrane.

The diameter of the pores in this membrane is approximately 6 μ . The diameter of the molecules in chilomonad growth substance is therefore less than 6 μ , i. e. probably less than that of molecules of serum albumin.

In preceding experiments it was demonstrated that the growth substance is heatlabile. Further experiments concerning its properties are in progress.

98. POPULATION FLUCTUATIONS IN PARAMECIUM CULTURES. Edgar P. Jones, University of Akron. (Lantern; 10 min.)

Data from several cultures of *Paramecium multimicronucleatum* have been collected at intervals of 4 hours for total periods exceeding $2\frac{1}{2}$ days.

The data indicates that population fluctuations (destruction followed by up-growth) are of daily occurrence. In a dense culture (300 to 1000 per cc.) the fluctuations are of such an order that $\frac{1}{4}$ to $\frac{1}{2}$ of the total population is oftentimes destroyed within 24 hours. This destruction does not leave dead bodies. However the turbidity of the culture changes. It is supposed that the mechanism of removal is by bursting.

A dense culture of *Paramecia* is therefore believed to be a system in which the tempo of the struggle for existence strikes an usually rapid rate.

An explanation of the causes of fluctuation is sought. It seems probable that an elaboration of a cytolytic agent is one of the variables.

99. CYTOLYZING ACTION OF TARANTULA VENOM. Edgar P. Jones, University of Akron. (Lantern; 5 min.)

A well-starved *Tarantula* seized at the throat a frog which was introduced as food. The frog tissues became deeply eroded within 3 hours. Histolysis was obvious for the white epithelial surface was entirely removed by digestion, as was much of the underlying muscular tissue. The digestion of tissue was sufficient, about the mouth, to expose the left aspect of the mandible.

The cytogenic agent is believed to be a portion of the fluid introduced by the chelicerae.

It therefore appears: (1) that the toxin may act as an exogenous digestive enzyme, or (2) that it is accompanied by such a substance.

100. INVESTIGATIONS CONCERNING SOME OF THE PROPERTIES OF THE NATURAL ACTIVATOR OF THE ENZYME TYROSINASE OF THE GRASSHOPPER EGG (*MELANOPLUS DIFFERENTIALIS*) WITH ESPECIAL REFERENCE TO THE EFFECTS OF TEMPERATURE. J. H. Bodine and L. D. Carlson, State University of Iowa. (Lantern; 7 min.)

The properties of the natural activator have been investigated both in a brei preparation and in an emulsion prepared in buffered NaCl. The brei preparation is made from 3-day prediapause eggs since these eggs contain no tyrosinase and are the most potent source of the activator. The emulsion is prepared using activator (the lipoidal layer of the centrifuged brei) from the same source and in concentrations as nearly identical to those found in the brei as possible.

The brei is prepared at 25°C., subjected to various temperatures for different periods, brought to 25°C. and its activity determined. Temperatures below 25°C. have no effect on the activator. Between 25°C. and 100°C. the activity of the brei is progressively depressed; being almost completely destroyed at 100°C. Curiously, the effect of temperature on the brei does not change with varying lengths of exposure.

The emulsion of the activator in NaCl is similar in its action to the brei preparation but is not affected by heat. A mixture of the NaCl emulsion with a brei from which the activator has been removed is affected by heat in the same manner as the activator in brei. When brei minus the activator is heated and then mixed with the emulsion, reduction in activity also occurs.

101. CYTOCHROME OXIDASE DURING THE DEVELOPMENT OF THE GRASSHOPPER EGG. T. H. Allen, State University of Iowa. (Introduced by J. H. Bodine.) (Lantern; 8 min.)

When an excess of cytochrome c extracted from beef hearts according to the method of Keilin and Hartree, together with 0.033 mM. of p-phenylenediamine is added to preparations of fifty eggs of the grasshopper, *Melanoplus differentialis*, at a pH = 7.15 and a temperature of 25°C., the velocity of oxygen uptake over and above that of a similar system in which the oxidase preparation has been heated for 10 minutes at 85°C. appears to be directly related to the inherent amount of cytochrome oxidase. With this material there are two separately located factors which for the interpretation of cytochrome oxidase are extraneous. The first of these is associated with the shell-like chorion membrane and may be removed by dechorionation of eggs or by low speed centrifugation of an egg brei. The second of these is found in the dechorionated egg or in the more supernatant fluid along with the cytochrome oxidase. Its existence may be ascertained by its heat stability. Both factors are heat stable (85°C.) and unable to catalyze the oxidation of reduced cytochrome c but do promote the oxidation of p-phenylenediamine alone or in the presence of cytochrome c.

The cytochrome oxidase is apparently absent during the first 10 days of the prediapauses period but later increases to only "2 to 4 mm³ oxygen uptake in the initial ten minutes" of a reaction. This same rather small amount is present throughout diapauses and increases linearly and over tenfold by the end of the postdiapauses period.

102. THE STABILITY OF THE DIAPAUSES CONDITION IN EGGS OF THE GRASSHOPPER, *MELANOPLUS DIFFERENTIALIS*. W. A. Robbie, State University of Iowa. (Introduced by Titus Carr Evans.) (Lantern; 7 min.)

The stability of the diapauses state in the egg of the grasshopper, *Melanoplus differentialis*, has been studied by subjecting eggs to various experimental conditions, including centrifugation, drying, temperature of 10° and 36°C., and atmospheres of nitrogen, oxygen and carbon dioxide, and noting the number of individuals that start active development after the treatment. The stability of the diapauses state varied with the age of the eggs, the younger ones being relatively resistant to all the treatments used, while the older ones were more easily in-

duced to resume growth. In eggs that were 160 days old a maximum of 65% came out of the diapause blocked condition after drying and rehydration. Other effective treatments on eggs which had been in diapause for a relatively long time were centrifugation, exposure to nitrogen, and temperatures of 10° and 36°C. Exposure for 6 days to 100% oxygen, carbon dioxide, and hypertonic sodium chloride solutions resulted in a smaller number of eggs breaking diapause than in the untreated control groups.

103. MAXIMUM RATES OF OXYGEN TRANSPORT FOR CERTAIN FRESHWATER FISH.

Edgar C. Black, F. E. J. Fry and W. J. Scott; Swarthmore College and University of Toronto. (Lantern; 15 min.)

Experiments have been performed with a respiration chamber on a turntable in which fish swim against the current generated in the rotating chamber. The maximum sustained rate of oxygen consumption is attained when these fish are still swimming slowly (peripheral velocity of the chamber 1 foot per second). Under the conditions of the test the maximum oxygen consumption of the catfish was greater than that of the perch. These tests showed that the maximum rate of carriage of oxygen did not vary to the extent that might be predicted from the effect of CO₂ upon the unloading of oxygen from the blood. Moreover, such variation as was observed in the maximum transport of oxygen by the exercised fish did not correlate with the CO₂ effect upon the oxygen combining power of the blood.

104. THE EFFECT OF CARBON DIOXIDE ON THE RATE OF OXYGEN UPTAKE BY FRESH-

WATER FISH. F. E. J. Fry, Edgar C. Black and W. J. Scott; University of Toronto and Swarthmore College. (Lantern; 15 min.)

The rate at which fish removed oxygen from a closed bottle of water has been measured and the influence of CO₂ upon the uptake of oxygen has been determined. The fish employed were the common sucker, sunfish, carp and catfish. As the fish used oxygen from the closed bottle the pressure of oxygen was diminished and the rate of the utilization of oxygen decreased. The presence of CO₂ in the water caused an initial decrease in the rate of the utilization of oxygen as compared with the rate in the absence of CO₂. The decrease in the rate of oxygen consumption was proportional to the pressure of CO₂. In the presence of CO₂ the rate of uptake of oxygen persisted undiminished in spite of a fall in the pressure of oxygen until the pressure of oxygen reached a low value, for the utilization of oxygen was independent of the pressure. The change in rate of oxygen uptake induced by a given pressure of CO₂ varied with the size of the CO₂ effect upon the oxygen combining power of the blood of each species.

105. THE MECHANISM OF DEATH BY COLD IN THE MYCETOZOA. Sister M. Pierre

Gehenio and Basile J. Luyet, St. Louis University. (Lantern; 8 min.)

The homoiotherms and certain plants are known to die by the action of cold, above 0°C. Death in these highly differentiated forms is usually attributed to a disturbance of the balance between the various interrelated physiological functions. But we have found that one of the least differentiated forms of protoplasm,

that of the mycetozoa (*Physarum polycephalum*), in the plasmodial stage, is also killed by cold without ice formation, either above its freezing point ($-0.17^{\circ}\text{C}.$) or in the subcooled state. Our criterion of death was the subsequent complete disintegration of the protoplasm. Either abrupt or gradual cooling was lethal. With an abrupt cooling, the time required to kill was remarkably short, of the order of 5 seconds at 0° . With lower rates of cooling, the lethal time was increased. The changes leading to death during a gradual temperature lowering from room temperature to about -5° , at the rate of 1 degree per minute, were studied microscopically during the cooling process. A phase separation accompanied by a breakdown of the pigment granules could be observed. If cooling was stopped at any stage during this process, the portions which had undergone phase separation were dead; the other portions recovered completely. The phase separation responsible for death is, in many respects, identical with syneresis in colloids.

106. SOME PROPERTIES OF VITRIFIED MUSCLE FIBERS. G. Thoennes and B. J. Luyet, St. Louis University. (Lantern and Motion picture; 7 min.)

As reported previously, isolated fibers of frog's muscle do not lose their power of reacting to electric stimuli after rapid cooling by immersion in liquid air (-195°) and subsequent rapid warming by immersion in warm Ringer's. The rapidity of the temperature change has for its purpose to carry the tissue across the zone of crystallization temperatures which extends some tens of degrees below $0^{\circ}\text{C}.$ in less time than necessary for formation of ice crystals. The material solidifies in the vitreous state, that is, with the least possible molecular rearrangement. A microscopic study revealed little difference between the vitrified and the normal fibers. A physiological investigation brought out the following points: 1. The average number of responses to repeated stimulation was found to be about 20 in the vitrified fibers and about 250 in normal fibers; 2. After their maximal number of contractions the vitrified fibers went into rigor, while normal fibers did not go into rigor and were able to respond to stimulation after 5 to 10 minutes in aerated Ringer's; 3. The permeability of the vitrified fibers was altered. While, in normal fibers, the increase in width due to the absorption of water in a hypotonic solution reached about 8% of the original, in vitrified fibers the maximal increase was only about 3%. The results obtained indicate that while the vitrification procedure does not kill muscle fibers, it does injure them. The injury is probably due to imperfect vitrification, that is, to the formation of crytoerysals of water.

107. TEMPERATURE ACCLIMATION AND ACCLIMATIZATION IN CLADOCERA. Katherine K. Sanford and A. M. Banta, Brown University. (Lantern; 15 min.)

Parallel cultures, derived in each case from a single individual, of *Moina macrocopa* and *Daphnia longispina* were maintained solely by parthenogenesis for a period of several generations at 10 to $17^{\circ}\text{C}.$ (usually about $15^{\circ}\text{C}.$) and at about $31^{\circ}\text{C}.$ From time to time individuals of the two temperature groups were tested for temperature tolerance. For test a group of animals (usually 10) in the second adult instar was placed in a large test tube in medium (5 cc.) at laboratory temperature. When parallel groups were ready the test tubes in a rack were immersed in boiling water until the temperature within the test

tubes reached about 44°C. when the rack was transferred to ice water until the temperature returned to the original level. For both species there was a significantly greater survival among those reared at the higher temperature.

When *Moina macrocopa* previously acclimated to one temperature were reared for a single generation at the other temperature they exhibited no difference in temperature tolerance from individuals continuously reared for several generations at the latter temperature. This test was not made was *D. longispina*.

Thus acclimation was indicated but there was no evidence of acclimatization.

108. PANTING AND TEMPERATURE REGULATION IN THE CHICKEN, *GALLUS DOMESTICUS*. W. A. Hiestand and W. C. Randall, Purdue University. (Lantern; 10 min.)

Panting in the chicken as a response to rising body temperature was demonstrated in this investigation. That panting is regulated by a center separate from the respiratory center is indicated by the use of centrally acting drugs. The critical panting temperature varies with individual birds as well as with the state of activity of the central mechanism. Reflex inhibition of panting following external chilling invariably occurred even though no appreciable drop in internal temperature was noted. Reflex induction of panting was obtained in only one of thirty-three experiments. Above a certain upper critical temperature level the panting mechanism begins to fail, resulting in a reduced panting rate. The upper lethal body temperature was found to be 47°C. Central depression (nembutal) reduces panting rate in proportion to the depth of narcosis. Light anesthesia raises the panting temperature threshold; deep anesthesia abolishes panting entirely. Depressant drugs affect the panting center independently of the respiratory center. Central stimulation (lobeline) results in a prompt but short-lived increase in rate and amplitude of respiratory movements followed by a longer period of increased sensitization of the panting mechanism. This sensitization is apparent in a lowered panting threshold, an increase in panting rate in relation to internal temperature, and a greater maximal panting rate.

109. GROWTH RATES IN THE LARVA OF THE MEXICAN BEAN BEETLE, *EPILACHNA VARIVESTRIS* MULS. Nellie M. Payne, American Cyanamide Company. (Lantern; 15 min.)

Accompanying increase in size of the larva of the Mexican bean beetle is the marked change from campodiform to eruciform. This is brought about by (1) a steep growth gradient with fastest rate in the mid dorso-lateral plane and (2) by overgrowth of the head by the prothorax. Growth in width accelerates and growth in length decelerates relatively. Associated with changes in external form are changes in the arrangement of internal organs and in the ratio of fat to water.

110. RELATIVE GROWTH IN THE HOUSE WREN, *TROGLODYTES DOMESTICUS BALDWINI*. Sara E. Huggins, Western Reserve University. (Introduced by A. H. Hersh.) (Lantern; 15 min.)

Data on the growth of nestling house wrens are analysed with respect to the relative growth formula, $y = bx^a$. In all cases x represents total length and y represents the length of the region under consideration.

Double log plots for some of the regions are best fitted to one straight line and some are better fitted to two, with the break occurring on the eighth day. Little significance is attached, however, to relative growth constants beyond the eighth day because all regions are approaching the upper limits of their growth cycles.

The values of α along the main body axis are: bill, 1.3205; head, 0.6988; neck, 0.9599; body, 1.0167; tail, 1.0551. This is a definite postero-anterior gradient with the exception of the bill. It has the high growth rate characteristic of late arising structures.

The leg shows a disto-proximal gradient with values of α as follows: femur, 1.2863; tibio-tarsus, 1.4106; tarso-metatarsus, 1.5778; middle toe, 1.5688; nail, 2.2190. This is similar to the gradient found by other workers in birds and mammals.

The values of α for the wing segments are: humerus, 1.3270; radius-ulna, 1.4914; manus, 1.2150. This growth pattern is dissimilar to that found among mammals but there is a suggestion of a common specialized pattern of growth among birds.

Relative growth constants for individuals vary considerably from the average and the gradients are not always the same. The gradient in the leg is much more constant among individuals than that along the main body axis.

111. A CYTOLOGICAL STUDY OF COELOSPORIDIUM PERIPLANETAE. Victor Sprague, University of Illinois. (Introduced by R. R. Kudo.) (Lantern; 7 min.)

Coelosporidium periplanetae is a haplosporidian which passes through its entire life-cycle living free in the lumina of the Malpighian tubules of *Blatta orientalis* and other cockroaches.

The trophozoite is an amoeboid body attached to the walls of the Malpighian tubules. It contains numerous small, massive nuclei. There may also occur few to many chromatoid bodies of various sizes. The latter have often been regarded as large nuclei but this view is not confirmed by Feulgen's nucleal reaction.

Eventually the small massive nuclei of the plasmodium become transformed into large vesicular nuclei, each of which becomes the nucleus of a young spore. During subsequent development the nucleus undergoes a process which, after hematoxylin, resembles autogamy but which actually is fundamentally different as revealed by Feulgen's method. The nucleus of the young spore constricts in a plane perpendicular to the long axis of the spore and produces two daughter nuclei which do not completely separate but lie in close approximation. The membranes of these nuclei break down permitting the extrusion of certain metabolic products having a strong affinity for hematoxylin. They have been previously regarded as products of a second (presumably reduction) division; but this view is unconfirmed since the bodies are Feulgen negative. Subsequent development is a process of reorganization wherein the discarded material is absorbed by the cytoplasm, the nuclear membranes are restored and the two nuclei fuse to produce, in the mature spore, a single vesicular nucleus.

112. OBSERVATIONS ON THE NUCLEUS OF *ENDAMOEBA BLATTAE*. Paul A. Meglitsch, Wright Jr. College, Chicago. (Introduced by Waldo Shumway.) (Lantern; 10 min.)

The nucleus of *Endamoeba blattae*, the large amoeba inhabiting the colon of the cockroach, has been studied with basic dyes and with the Feulgen nucleal reaction. The endosomes and peripheral granules which occur in the peripheral region of the nucleus are stained with basic dyes but do not react with the Feulgen reagents. Chromosomes, the only Feulgen-positive structures in the nucleus, appear during division and develop from material which does not stain deeply with the basic dyes. The intensely basophilic endosomes undergo a complete reorganization during each division of the trophic nucleus, and appear to be negative to the Feulgen reaction even during division. During early precystic divisions the nucleus undergoes a reduction in the amount of endosomal and peripheral material and proceeds to a far-reaching reorganization of nuclear morphology. This structure is maintained until the cyst membrane is formed, when a second change in the nuclear structure occurs. As a result of these nuclear transformations the Feulgen-positive material comes to coincide with the little basophilic material remaining in the nucleus. These observations, leading to a number of interesting questions concerning the nature of chromatin in the Sarcodina, have been accompanied by attempts to utilize the suggested specific chromatin tests, with the result that a wide divergence of results were obtained from different tests. Nuclear structure seems to clearly indicate a close relationship of *Endamoeba blattae* to certain amoebae found in termites.

113. A CYTOLOGICAL STUDY OF *BLEPHARISMA* WITH SPECIAL REFERENCE TO ITS FIBRILLAR STRUCTURES. C. Leplie Kanatzar, University of Illinois. (Introduced by R. R. Kudo.) (Lantern; 8 min.)

A fibrillar system has been demonstrated in *Blepharisma persicinum*, a heterotrichous ciliate, through observations on the living organism and the silver technique of Gelei and Horvath, and includes: a) undulating membrane strand and undulating membrane, b) band of membranelle plates and membranelles, c) inner and outer membranelle fibrils, d) pharyngeal complex of cytopharynx, two pharyngeal membranelle fibrils, membranelles, and oesophageal fibrils, e) longitudinal ciliary fibrils, and f) body cilia and their basal granules. The system primarily coordinates the locomotor organelles and ingestatory activity, secondarily serves for contraction and support, and possibly possesses a metabolic function. Coordination is indicated by a) morphological relationships, b) affinity of fibrils for acid fuchsin and silver, c) an axial gradient of the type expected if the system is conductile, and d) comparison with metazoan nervous tissue. Four movements of the organism are described: a) swimming, b) forward movement without rotation, c) avoiding reaction (movement posteriorly), and d) circling movement. Chlorides are necessary for silver impregnation of fibrils. Unchlorinated tap water is substituted for distilled water which removes chlorides.

114. GENETIC EVIDENCE OF AUTOGAMY IN *PARAMECIUM AURELIA*. T. M. Sonnenborn, Indiana University. (Lantern; 15 min.)

Nuclear reorganization in *Paramecium aurelia* is generally held, on cytological evidence, to be a simple replacement of macronucleus by micronucleus without chromosome reduction or fertilization ('endomixis', Woodruff and Erdmann and others); but Diller maintains the process involves reduction and fertilization ('autogamy'). These alternatives were tested genetically in variety 1 of *P. aurelia* by determining the genotypes following reorganization in clones of genotype Aa (genes determining mating types). The reorganizations occurred under four diverse sets of conditions: (1) in mass cultures at 31°C., to test induced reorganizations; (2) in isolation lines at 27°C., to test natural reorganizations under standard cultural conditions; (3) in isolation lines at room temperature in unboiled pond water as culture fluid; and (4) in isolation lines at room temperature in pond water used 24 hours after boiling. The latter (3 and 4) were employed to simulate as closely as possible the conditions under which others have reported endomixis. Genetic analysis of the reorganizations under conditions (1) and (2) shows that all resulting lines are homozygous, half of them dominant, and half recessive. From any one reorganizing individual both caryonides are of the same genotype. Thus, under these conditions, autogamy, not endomixis takes place. (In these experiments 122 reorganized lines were examined: in 36 the genotypes were determined and in the remainder merely the phenotypes.) Analysis of the reorganizations under conditions (3) and (4), still in progress, will be completed and fully reported. Autogamy appears also to occur under these conditions.

115. FURTHER OBSERVATIONS UPON SEXUAL DIFFERENTIATION IN *VORTICELLA MICROSTOMA*. Harold E. Finley, Morehouse College. (Introduced by Lowell E. Noland.) (Lantern; 10 min.)

The author previously reported evidence of sexual differentiation in this peritrichous ciliate. The previously reported investigation was confined to vorticellae descended from a West Virginia stock; therefore, a study was made to determine: 1) whether non-conjugating stocks could be discovered in populations of *Vorticella microstoma* from diverse localities; 2) whether West Virginia stocks could be induced to conjugate with stocks from other localities.

Collections were made in different places in the area of Atlanta, Georgia. Two Georgia stocks (CL and LP) were subsequently established from wild populations and many strains were isolated from these stocks. The same evidences of sexual differentiation previously reported from West Virginia stocks were found in the Georgia stocks. Inter-stock conjugation was induced by synchronizing epidemics of conjugation in the three stocks. That is to say, conjugation occurred when microconjugants from the West Virginia stock were placed in a small volume of culture medium which contained macroconjugants from either the Georgia CL stock or the Georgia LP stock. The reciprocal experiments yielded similar results; inter-stock conjugation occurred when macroconjugants were taken from the West Virginia stock and exposed to microconjugants from either of the Georgia stocks.

These observations do not preclude the possibility of discovering non-conjugating stocks in this species but they seem to strongly suggest the other point of view. Their significance in the problem of sexual differentiation in *Vorticella microstoma* will be discussed.

116. MICROSCOPIC ANATOMY AND REGENERATIVE BEHAVIOR OF A COMPLEX HETEROTRICHOUS CILIATE, *LICNOPHORA MACFARLANDI*. William Balamuth, University of California and University of Missouri. (Introduced by W. C. Curtis.) (Lantern; 15 min.)

Licnophora macfarlandi is a highly organized ciliate, inhabiting commensally the respiratory tree of *Stichopus californicus*. The body is differentiated into a membrane-bearing basal disc adapted for attachment, an erect oral disc equipped with adoral zone and cytostome, and an intermediate neck-like region. The nuclear apparatus consists of a single basal micronucleus and a string of macronuclear beads.

Cytological investigation clearly justifies the view that *Licnophora* is one of the more complex *Heterotrichina*. The internal fibrillar apparatus is exceedingly complex and unites all ciliary organelles on the body into a structurally coördinated system.

Removal of part of the adoral zone results in a profound reorganization involving the entire oral region. New organelles arise from a localized zone along the left side of the body, and subsequently migrate to their definitive positions. If this zone is removed, a new anlage arises alongside.

Nuclear reorganization has been followed. Regeneration occurs only in the presence of macronuclear substance, the micronucleus being dispensable for the morphogenetic process.

In general, the regenerative response resembles that of similarly treated *Heterotrichina* (*Stentor*, etc.). Furthermore, it is strikingly similar to fission in *Licnophora*.

Regulation and regeneration follow various types of operations excepting the removal of the basal disc. This failure seems to be correlated with the origin of this structure during fission only by constriction of the parent structure and never by duplication. This result lends support to a view (corroborated by the literature) that regenerative capacity in Protozoa involves at most a realization of normal developmental potentialities.

117. EFFECTS OF MECHANICAL AGITATION ON *PARAMECIUM CAUDATUM*. R. L. King and H. W. Beams, State University of Iowa. (Lantern; 15 min.)

Mechanical agitation of *Paramecium caudatum* results in a decrease in locomotor activity accompanied by a significant decrease in the rate of food vacuole formation. Not only are fewer food vacuoles formed but usually there is less ingested material (India ink or carmine) per vacuole. Mechanical agitation is also followed by an increase in the rate of cytoplasmic rotation, as measured by movement of food vacuoles, and by changes in refractive index whereby the macronucleus and micronucleus become very clearly visible. Long continued jolting eventually causes the breakdown of the protoplasm often leaving only the pellicle intact. In some cases the macronucleus becomes scattered about the cytoplasm or may be completely destroyed before solution of the protoplasm.

118. THE EFFECT OF SUB-NARCOTIZING AND NARCOTIZING CONCENTRATIONS OF FOUR PRIMARY ALCOHOLS ON STARCH PRODUCTION IN *CHILOMONAS PARAMECIUM*. Joseph F. Oliphant, Stanford University. (Introduced by Willis H. Johnson.) (Lantern; 10 min.)

Sub-narcotizing concentrations of ethyl alcohol, 1/50% to 2% resulted in increase in starch production and in volume up to 100% in *Chilomonas paramecium* cultured on a variety of media. Sub-narcotizing concentrations of methyl, isopropyl, and N-butyl alcohols had no effect on starch production but narcotizing concentrations of all the alcohols inhibited starch production, the degree of inhibition varying with the concentration of alcohol. The narcotizing action of the alcohols is probably associated with their action on the surface membrane of the cell. The cell membrane of *Chilomonas* loses its selective permeability and the organisms die immediately when the surface tension at the air medium interface is lowered to 70% that of distilled water. Recovery of narcotized organisms is correlated with an increase in surface tension at the air medium interface to 82% that of distilled water. The inconsistent and contradictory results of studies on the effects of alcohols on the protozoa are perhaps to be expected, especially because of the variability of the media employed and conditions of the experiments. The results of the present investigation suggest that many of the effects reported for alcohols are secondary and indirect.

119. THE INFLUENCE OF FOOD ON THE STRUCTURE OF A NEW SPECIES OF *GLAUCOMA*. George W. Kidder, Brown University. (Lantern; 15 min.)

A hitherto undescribed species of *Glaucoma* was isolated from nature, established in pure culture and tested for food requirements. It was found that the shape and size of the ciliate was conditioned by the type of food present in its environment, previous to its ingestion. In broth cultures (proteose-peptone, yeast extract, etc.) the ciliates are able to utilize the dissolved proteins and increase in numbers to relatively high concentrations. These saprozoic forms are small and ovoidal. When feeding on bacteria (as in nature) they are very elongate and possess a tail. This same form is retained when the ciliates are fed dehydrated yeast cells (Yeast Harris) in sterile suspensions. Autoclaved *Colpidium* supports very good growth in sterile culture, the *Glaucoma* ingesting the cells and cell fragments. They increase appreciably in size and become very dark. The form of the dead *colpidia*-feeders is bluntly tailed. When placed in a culture of sterile, live *Colpidium* the *Glaucoma* become carnivorous. Before they can ingest the living *colpidia*, however, form changes are necessary. These changes involve the formation of a huge vacuole back of and continuous with the mouth, for the reception of prey, enlargement of the mouth opening and a swelling of the body of the *Glaucoma*. The general form of the carnivore is that of a teardrop with the posterior end rounded. Other species of ciliates will also evoke the above response. Living flagellates and yeast fail to evoke the response, nor will extracts of ciliates. After the population becomes dense in pure cultures in broth cannibalism results, the cannibals going through the same form changes as noted for the *colpidia*-feeders.

120. THE FEEDING MECHANISMS OF VARIOUS CILIATED PROTOZOA. Everett E. Lund, Alfred University. (Lantern; 12 min.)

The nature and the activity of the various organelles primarily responsible for the ingestion of solid food is considered in detail for *Paramecium*, *Oxytricha* and *Stylonychia*.

In *Paramecium* the food vacuoles are not liberated at the extreme tip of the pharyngo-esophageal apparatus as is commonly supposed, but from a region somewhat anterior to this at the aboral surface of the esophagus. Posterior to this region is a pocket, the esophageal process, which is heavily ciliated on its oral surface. These cilia beat with their effective stroke directed anteriorly in such a way as to concentrate the solid food at the point where the vacuole will be formed. Other organelles, some of which beat posteriorly, aid in this concentration. Food vacuoles are liberated into a region of protoplasm of low viscosity in which several long fibrils of two general types undulate. Trapped in these undulating fibrils the vacuoles are started on their way at a relatively rapid rate, but on passing beyond the fibrils the progress is much slower.

In *Oxytricha* and *Stylonychia* the food concentrating mechanisms differ in many respects from that in *Paramecium*, but the undulating fibrils for conducting vacuoles into the cytoplasm show surprising similarities in both structure and behavior.

Many ciliates have no postesophageal fibrils; and some of these forms are very much less efficient in ingesting small particles than are those forms having such fibrils. However, diatoms and other moderately large particles are taken with ease.

121. GROWTH IN VOLUME IN THE CILIATE *ICHTHYOPHTHIRIUS*. R. F. MacLennan, State College of Washington. (Lantern; 10 min.)

A uniform group of parasites was obtained by exposing clean fish of uniform size to infective ciliospores for 15 minutes. The parasitized fish were kept in an aerated aquarium maintained at 22°C. A fish was killed every 12 hours over a period of 120 hours, the parasites freed and killed in 1% formalin. The volume of each parasite was calculated from the diameter. This method is more satisfactory than measuring the living ciliates since these fluctuate in volume after being freed and attain a constant volume and shape only when encysting. Experiments showed that after being killed in 1% formalin the ciliates become spherical and take on the volume they would have if encysting with an error of less than 5%. This method makes possible the use of not only mature ciliates but also the younger ciliates which are not able to encyst.

The logarithm of the mean volume at each interval was plotted against time. These points are in a linear position indicating a constant proportional growth rate of 8% per hour at the temperature used. No change in the growth rate could be demonstrated at 72 hours although in view of the profound physiological changes at this time in connection with the onset of maturity, it would be reasonable to expect an inflection in the growth curve at that time.

122. EFFECTS OF IODINE ON GROWTH OF THE FLAGELLATES, *ASTASIA* AND *EUGLENA*. J. B. Loefer, H. W. Schoenborn and R. P. Hall, Berea College and New York University. (Lantern; 15 min.)

Iodine, in concentrations ranging from 20 to 0.025 parts per million, was added to a medium containing casein-peptone (0.5%) and certain inorganic salts. After 5 days of incubation, growth of *Astasia* sp. was accelerated from 30% to about 130% in different concentrations of iodine, as compared with iodine-free controls. After 8 days, acceleration ranged from approximately 30% to 400%. In *Euglena gracilis*, growth at 5 days of incubation was accelerated from 25% to more than 400% in different iodine concentrations; after 8 days the acceleration ranged from about 25% to more than 1300%. Maximal effects on *Euglena* were noted in 5 to 20 ppm of iodine; on *Astasia*, in 1 to 5 ppm. In a simpler medium containing twice as much casein-peptone, the accelerating effect of iodine was slight for both species.

123. POPULATION STUDIES ON PURE CULTURES OF A COLORLESS FLAGELLATE, *ASTASIA KLEBSII*. Herman Von Dach, Ohio State University. (Introduced by R. C. Osburn.) (Lantern; 8 min.)

Bacteria-free clone cultures of *Astasia klebsii* in 0.5% tryptone showed good growth within the range pH 3.2 to 7.0, with maximum growth between pH 5.1 and 6.0. Addition of 0.2% sodium acetate had little effect at pH 7.8, but greatly increased growth at initial pH 5.9. In acetate-tryptone at initial pH 5.9, both nearly-anaerobic cultures and constantly-aerated cultures showed lower growth rates but longer growth periods than the controls. The pH of the tryptone cultures remained fairly constant. In vigorously-growing cultures in acetate-tryptone a marked increase in pH occurred; this was probably due to cellular oxidation of the acetic acid, with resultant accumulation of sodium hydroxide.

Growth curves of this organism were determined, and the effects of various factors upon various phases of the growth curve were studied.

124. THE MECHANISM OF FLAGELLAR AND CILIARY MOVEMENT. Bruce D. Reynolds, University of Virginia. (Lantern; 12 min.)

Exception is taken to the generally accepted opinion that movement of flagella and cilia is caused by the active contraction of the axial fiber. Evidence, supported by photomicrographs, will be presented to show that the axial filament serves as a straight, or spiraled, thickening of the ectoplasmic tube which causes the latter to bend in the direction of the thickening as a wave of contraction passes from its basal toward its apical end. Recovery is probably due to the elasticity of the wall of the tube.

125. RELATION BETWEEN CENTRIOLE AND CHROMOSOME NUMBERS IN ATYPICAL SPERMATOGENESIS OF *VIVIPARUS MALLEATUS* REEVE. Arthur W. Pollister and Priscilla Frew Pollister, Columbia University. (Lantern; 15 min.)

Atypical spermatogenesis of *Viviparus* (Paludina) has always been considered to involve multiplication of centrioles without nuclear division. Study of the Japanese aquarium snail shows 18 centrioles (two groups of 9) in primary

spermatocytes—16 more than the typical normal number. In secondary spermatocytes two chromosomes behave normally, one going to each polar group of centrioles and forming a nucleus in each spermatid. The number of chromosomes at poles of Division I likewise indicates that of the total of 36 chromatids ($N=9$) 4 are normal; the other 32 are abnormal, show no relation to spindles, and finally disintegrate. Bouquet orientation and synapsis are greatly reduced or absent. In *Amphiuma* Schrader has shown that each pair of sister chromatids, until middle diakinesis, contains a single centromere (kinetochore). Assuming the same in *Viviparus*, the 32 abnormal chromatids contain 16 centromeres. Schrader has called attention to morphological resemblances between centrioles and centromeres. Darlington holds that centrioles and centromeres are essentially similar bodies that behave differently because one is cytoplasmic, the other nuclear. Since, in *Viviparus malleatus*, the extra centrioles exactly equal the number of centromeres of chromosomes that act as if acentric (e.g. fail to show relation to spindle) it is suggested that the 16 extra centrioles have not arisen, as formerly believed, by multiplication of the original 2, but that they actually represent 16 centromeres that have become detached from 16 pairs of sister chromatids, entered the cytoplasm, and taken on centriole properties that were inherent in them but unexpressed while they were within the nucleus.

126. CHROMOSOME DIVISIONS IN THE NURSE CELLS OF THE OVARY OF *DROSOPHILA MELANOGASTER*. T. S. Painter and Elizabeth C. Reindorp, University of Texas. (Lantern; 15 min.)

During the growth of ova the nuclei of nurse cells increase in diameter from about 5μ to 40μ or more. This represents an 8-fold doubling of the nuclear volume. Nurse cell nuclei exhibit a great variety of chromosome configurations which we have been able to correlate with a series of intra-nuclear chromosome division cycles (endomitosis) which parallel the changes seen in ordinary mitosis from the resting to a late prophase stage when, in nurse cell nuclei, the chromosomes divide autonomously. There is no metaphase plate nor anaphase movement, no sign of a spindle and throughout the cycle the nuclear wall remains intact. Each cycle has a resting stage, when the chromosomes are evenly distributed about the nucleus, then a period of condensation (coiling) accompanied by an increase in the nucleic acid, as shown by the Feulgen test. In late prophases homologous chromatids tend to associate together and in smaller nuclei they may appear as large chromosomes; later there are discrete bundles of threads, six in number corresponding to the six large chromosomes of this species, and finally, in the largest nuclei, there are localized tangled masses of chromatids. The largest nuclei are at least 512-ploid. This study gives striking confirmation for the polytene or multiple strand concept of salivary chromosome structure.

127. EFFECTS OF 250 R OF X-RAYS ON NEUROBLAST MITOSIS IN THE EMBRYO OF THE GRASSHOPPER (*CHORTOPHAGA VIRIDIFASCIATA*). J. Gordon Carlson, University of Alabama. (Introduced by C. M. Pomerat.) (Lantern; 7 min.)

The average numbers of neuroblasts in each of several different mitotic stages were obtained from 63 untreated embryos as a basis for comparison with the

numbers after x-radiation with 250 r. Counts were made and averaged for 10 embryos fixed at each of several 15-minute intervals after x-raying.

Statistical and observational evidence indicates that middle prophase is the stage most susceptible to the x-ray effect that leads to cessation of mitosis; for after treatment some of the cells in this stage, instead of entering late prophase, revert to earlier stages or at least undergo changes in their chromatin that simulate reversion. Neuroblasts in this stage decrease gradually in numbers after treatment, disappearing after 75 minutes.

Late prophases and metaphases decrease steadily in numbers by passing into successively later stages until they have disappeared at the end of 75 minutes. Both anaphases and early telophases decrease in numbers by passing into successively later stages during the first 30 minutes, increase at 45 minutes, and then fall steadily, the former reaching zero at 90 minutes, the latter at 120 minutes. The simultaneous fall in the numbers of late prophase and metaphase stages and the increase simultaneously in the numbers of anaphases and early telophases at 45 minutes indicate that x-rays alter the rate of change from stage to stage in cells undergoing mitosis at the time of treatment and that this change in rate differs with the stage of mitosis and with the time elapsing after irradiation.

128. CELLULAR MOVEMENTS IN THE EMBRYO OF *DROSOPHILA MELANOGASTER*.

Alfred F. Huettnner, Queens College, Flushing. (Lantern; 15 min.)

After segregation from the blastoderm in the *Drosophila* embryo, the primordial germ cells remain attached to the posterior end in the perivitelline space. After a period of 70 minutes a complex series of cellular movements occurs which transfers the germ cells to the dorsal region of the embryo and pushes them into the gut. This process begins on the undifferentiated blastoderm on its ventral side by a longitudinal invagination of columnar cells, resulting in a longitudinal narrow cleft. The latter extends from approximately 60 to 70 μ behind the anterior tip of the embryo to the primordial germ cells. The slit closes immediately after its formation, thus forming a solid cylinder of cells attached to the ventral wall within the embryo. By changing their form from blastodermal columnar to irregularly shaped cylinder (mesentoderm) cells, this cylinder expands in its antero-posterior direction and pushes that part of the blastoderm to which the germ cells are attached, to the posterior region of the embryo. Another groove, referred to by some authors as the cervical or cephalic groove, constricts the blastoderm latero-ventrally, thus constricting the mass of cylinder cells so that they can expand only in the posterior direction, toward the germ cells. The so-called migration of the germ cells is therefore a simple mechanical process of expansion, conditioned by a change in cell form. This cellular movement and others following it, transfers approximately 25% of the superficial cells of the blastoderm into the interior of the embryo. This is of significance to experimental embryologists employing *Drosophila* as experimental material, since the inner cells play apparently no part in the external appearance of the imago.

129. ULTRACENTRIFUGATION OF RAT SPINAL GANGLION CELLS, WITH SPECIAL REFERENCE TO NEUROFIBRILLAE. H. W. Beams and H. W. Kirshenblit, State University of Iowa. (Lantern; 10 min.)

Spinal ganglion cells of the rat centrifuged at 400,000 times gravity for 30 minutes show a displacement of both the nucleus and the neurofibrillae to the centrifugal ends of the cells. The displacement of the neuro-fibrillae by the centrifuge is interpreted to support the view that they are real cytological structures rather than artifacts.

130. CYTOLOGY OF THE MAMMARY GLAND OF THE BAT MYOTIS GRISESCENS. Katharine R. Jeffers, Duke University. (Lantern; 12 min.)

The cytoplasmic inclusions of the cells of mammary glands of fifty bats killed during pregnancy, lactation and involution were studied. These inclusions are believed to be metabolic products accumulated in the cells and may be used as criteria of the activity of the gland.

Secretory cells from glands preserved late in pregnancy contain chondriosomes, numerous small fat drops and occasional pseudo-yolk spheres. Shortly before parturition there is an increase in pseudo-yolk in the cells. Discharge of fat, pseudo-yolk and protein from the cells into the lumen of the alveolus commences just prior to parturition and some nuclear degeneration occurs at this time.

Chondriosomes and fat drops occur in the cells of lactating glands. During lactation, fat may be discharged directly from the cell, or, more commonly, along with a mass of apical cytoplasm. Binucleate cells have been observed in glands from pregnant and lactating animals.

Nuclear degeneration is marked during early retrogression of the mammary gland. As involution progresses, the secretion in the lumina of the alveoli disappears and fat-laden cells appear between and in the alveoli. Chondriosomes and occasional small fat drops are found in the cells of the alveolar epithelium.

131. LYMPHATIC ORGANIZATION IN THE RABBIT APPENDIX. Edward D. Crabb, University of Colorado. (Lantern; 10 min.)

The wall of the adult rabbit appendix is composed chiefly of tall, primary lymph nodules. Each nodule is partially enveloped by a trabecular sheath, in which are large lymph sinuses and blood vessels, and is connected with the lumen of the appendix through a flask-shaped gland, the bottom of which is formed by the apex of the nodule covered by lymphoepithelium. Lymphatics arise as capillaries and sub-epithelial sinuses and continue along the trabeculae as sinusoidal vessels which empty into collecting ducts in the mesoappendix. In the apical third of the nodule is an area of active mitosis which is richly supplied with lymph capillaries.

The site of the developing nodule is readily recognized in 19-day post-coitus embryos as an aggregation of small lymphocytes in the basal stroma of specially aborted villi, the apical epithelium of which is columnar. Definite infiltration of the apical epithelium by lymphocytes occurred within 14 hours after birth and had formed a lymphoepithelium before the end of the first week. The nodular and vascular plan of relations had attained essentially adult form in 7-day young, but traces of villi persisted in 30-day old young.

132. THE TOTAL DISTRIBUTION OF TASTE BUDS ON THE TONGUE OF THE PUP. John C. Holliday, Ohio University. (Introduced by Rush Elliott.) (Lantern; 7 min.)

Data are presented on the distribution of taste buds in the fungiform papillae of the tongues of three pups 5 days old, and in the circumvallate papillae of one of the three tongues. The uneven number and distribution of the circumvallate papillae are shown for six tongues.

All taste buds observed on the tongue of the pup are found on the dorsum and here only in the fungiform and circumvallate papillae. No taste buds were observed caudad of the circumvallate papillae. An average of 1706 buds is present on a tongue. The highest number of taste buds observed in all the fungiform papillae in any tongue is 1774; 1444 being the average number in these papillae. In the fungiform papillae all taste buds are present in the tops. The circumvallate papillae of one tongue contained 262 buds. Seventy is the maximum number present in a single papillae, forty-four being present in the sides and twenty-six in the top.

A gradual shift for the acuity of the sense of lingual taste from the tip of the tongue toward the circumvallate papillae is observed.

133. DISTRIBUTION OF THE TASTE BUDS ON THE TONGUE OF THE KITTEN, WITH PARTICULAR REFERENCE TO THOSE INNERVATED BY THE CHORDA TYMPANI FIBERS OF THE FACIAL NERVE.—A PRELIMINARY REPORT. Russell Hayes, Ohio University. (Introduced by Rush Elliott.) (Lantern; 8 min.)

By cutting the lingual nerve, which carries the chorda tympani fibers of the facial nerve, in the kitten the taste buds on the homolateral side of the anterior part of the tongue were caused to degenerate. The remaining buds were counted and accurately located by projection and measurement of their positions, thus delimiting the area served by the intact nerve. The extent of the chiasma of the taste fibers was found to range from 1.3 mm. near the tip to 0.7 mm. near the region of the circumvallate papillae, with fibers reaching only to the midline in the region between these limits. The total count obtained in this area indicates an unexpected increase in the number of taste buds found in the 2-month-old kitten over the number found at birth. Positional relationships of the taste buds to the lingual papillae were studied. Degenerating taste buds on the unoperated side of the tongue were noted and studied, the cause and significance of this degeneration remaining obscure.

134. THE SNAKE EYE—AN EVOLUTIONARY 'COME BACK'. Gordon L. Walls, Wayne University College of Medicine. (Lantern; 15 min.)

The eyes of Sauropsida are all very similar except for the snakes, which, despite their close relationship to lizards, have eyes as different from lizard eyes as mammalian eyes are from amphibian ones. The snake eye is interpreted as a bundle of substitutes, and it is proposed that the snakes originated as burrowing forms whose eyes degenerated greatly, subsequently recovering by the production of ocular structural features which effectively replaced the lost ones which had been characteristic of the lacertilian ancestors.

135. MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES ON TWO SPECIES OF MARINE CATFISH, *BAGRE MARINUS* AND *GALEICHTHYS FELIS*. Daniel Merriman, Yale University. (Lantern; 15 min.)

Osteological studies show interesting differences between the two species, and demonstrate the high degree of specialization characteristic of the Nematognathi. This is exemplified by the cephalic and nuchal shields, the loss of various bones (e.g., subopercular), the extreme reduction of the maxillae, unusually large otoliths, close articulation of the pectoral girdle with the neurocranium involving the disappearance of the supracleithrum, Weberian ossicles, and the intricate articulation of the serrated pectoral spine with the cleithrum.

Both species show sexually dimorphic characters—e.g., the pelvic fins are smaller in the males. In *Galeichthys felis* the two sexes differ with respect to a remarkable hook-like protuberance on the adaxial surface of the pelvic fin. The gastro-intestinal tracts are similar in general pattern, the intestine in each describing one full loop and being highly convoluted. The length of the intestine is proportionately longer in *Bagre marinus* than in *Galeichthys felis*, since the convolutions always start nearer the pyloric valve in the former.

The eggs of both species are among the largest in the Teleosts. Those of *Galeichthys felis* average about 17 mm. in diameter. In the earliest stage so far seen, the eye and lens are clearly differentiated, the mesoderm is segmented, neuromeres are distinctly visible, and the otoliths have formed. This and the later stages through hatching up to the nearly complete absorption of the yolk sac have been subjects of study. During this entire developmental period the young are retained in the parent male's mouth.

136. STUDIES ON STARVATION IN LARGEMOUTH BASS. Marian F. James, University of Illinois. (Introduced by L. A. Adams.) (15 min.)

The purpose of this study was to determine the changes in weights and lengths of live largemouth bass, and changes in the weights and composition of the organs during complete starvation for 19 weeks.

Eighteen largemouth bass fingerlings (*Huro salmoides*) were used. The live fish were weighed and measured weekly by a standard technique. Each week one fish was chosen by lot, killed, and dissected under water by a uniform method. The wet, dry, and ash weights of the organs were obtained on a chainomatic balance.

An experiment to determine the effect of feeding on growth subsequent to a prolonged period of complete starvation was begun on two of the starved fish. One died but the other started to grow again.

During starvation there was a gradual decline in average live weight from 26.945 to 11.842 grams and in standard length from 108.72 to 103.69 mm.

The organs of the unstarved fish were used as 100% and the others calculated on this basis. Graphs of changes in wet, dry, and ash weights showed practically the same results. During the starvation, the wet weight of the eyes increased to 113%, the head decreased to 79%, the fins to 56%, the gills to 52%, the digestive tube to 49%, the trunk to 38%, the heart to 19%, and the liver to 16%.

The water content, the organic, and the inorganic substances in the different organs were also investigated.

137. A BASIS FOR A NATURAL CLASSIFICATION OF FIXING FLUIDS. Wilfrid T. Dempster, University of Michigan. (Lantern; 12 min.)

The common concepts that fixing fluids are balanced mixtures in which shrinkage and swelling effects are counteracted and that the fundamental characteristics of a fixing fluid depend upon specific corrosive or precipitating agents are easily challenged. When a tissue is immersed in a fixative, the ingredients of the latter penetrate into the tissue separately, each at its own rate and virtually uninfluenced by the other constituents of the fluid. Tests of the rate of penetration of fixing chemicals into a number of animal tissues may be expressed by exponential formulae, and, practically, the relative rates follow the sequence: acetic, formalin, trichloroacetic, sublimate, picric, bichromate, chromic, osmic. In view of the penetration by advancing fronts of each ingredient of a mixture, sequence of action becomes a keynote in the behavior of fixatives. The first penetrative acts on living tissue, the second on the product of this reaction, the third on the product of the second reaction, etc. If the first, or first and second, penetrative of two mixtures is the same, basic similarity of reaction is to be expected, but with modifications induced by the addition of unlike reagents. Concentration of individual reagents may be adjusted in fixing mixtures to alter penetration sequence. A classification based upon constituents in the order of penetration (with consideration for concentration) permits a culling of formulae from the technic handbooks, emphasizes the basic similarities and differences in mixtures and provides a background for comparison of staining and analysis of shrinkage effects.

138. STUDIES ON THE INFILTRATION OF MICROLOGICAL IMBEDDING MEDIA. Robert T. Hance, Duquesne University. (Lantern; 5 min.)

The penetration phenomena of a number of paraffin imbedding mixtures as shown under normal and varied atmospheric pressures will be discussed and illustrated by slides and later demonstrated in actual operation.

139. MICROSCOPICAL CHARACTERISTICS OF SOME IMBEDDING MEDIA. Robert T. Hance, Duquesne University. (Lantern; 10 min.)

Variations in the characteristic crystalline structure of pure paraffin as introduced by the addition of other paraffin miscible substances will be discussed and illustrated.

140. ON A THERMODYNAMIC CONCEPT OF THE ENVIRONMENT. F. H. Pike, Columbia University. (10 min.)

Bergson's statement "life is possible wherever energy descends the incline indicated by Carnot's law and where a cause of inverse direction can retard the descent," or some part of it, has sometimes been quoted uncritically. Hopkins states that "The arrest of energy degradation in living nature is indeed a primary biological concept." Retardation of the descent of energy by any cause outside of organisms usually means design or vitalism; hence, Bergson's *elan vital*. No permanent arrest of processes of degradation of energy is possible in living

organisms; but retardation of descent by living organisms seems a biological verity. Must the biologist accept vitalism, or is the process a natural process? To me, *elan vital* seems unnecessary.

Bergson's idea has, however, important fundamental implications in that it suggests description of the environment in thermodynamic terms. Gibb's general thermodynamic equations give a method for a complete description of the environment, including the quantitative relations of necessary substances, volume, pressure and temperature, optima and limiting factors, and the direction in which changes are possible.

Bergson recognized clearly the possibility of expressing certain relations of organisms to environment in thermodynamic terms. Individuality in living organisms rests upon a physical concept. While it is impossible to tell what is an individual, "life nevertheless manifests a search for individuality, as if it strove to constitute systems naturally isolated, naturally closed." Divested of *elan vital*, this is a thermodynamic concept. Degrees of freedom give the necessary physical basis for adaptation.

141. EGG TEMPERATURES OF WILD BIRDS UNDER NATURAL CONDITIONS. Russell A. Huggins, Western Reserve University. (Introduced by J. Paul Visscher.) (Lantern; 15 min.)

Egg temperatures were obtained for 37 species of birds including members of eleven orders.

Two instruments, the recording potentiometer and the galvanometer with copper constantan thermocouples, were used to measure the egg temperatures.

The average incubation temperature for all birds studied was found to be 34.15°C. The average for passerine birds was 33.61°C., and for the galliform birds 36.35°C.

A wide range of egg temperatures was found in nests in which the eggs are known to have hatched. A robin's nest showed a difference of 27.00°C. between maximum and minimum temperatures.

The location of the egg in the nest was found to be important. A mallard nest with 18 eggs showed a difference of 12.22°C. between an egg in the center of the nest and one at the outer edge.

A correlation of $r = +0.4335 \pm 0.09683$ was found between average attentive egg temperatures and air temperature. Other correlations of egg temperatures with air temperatures were inattentive period $r = +0.6642 \pm 0.0688$, average egg temperature $r = +0.4954 \pm 0.0706$, and maximum egg temperature $r = +0.4675 \pm 0.0980$.

Direct sun and exposure to wind were found to exert a very noticeable effect on egg temperatures.

142. DECEPTIVE APPEARANCE AS A FACTOR IN TROPICAL ECOLOGY. Theodore H. Eaton, Jr., Union College. (Introduced by James W. Mavor.) (15 min.)

The problem of deceptive appearance among animals may be approached by field observations covering the cases of actual deception in a single ecological association. A sample analysis of such observations, made in the rain-forest of Barro Colorado Island, Canal Zone, shows the following types of deception (from the

human standpoint): invisibility by transparency, misleading ('ruptive', etc.) markings, obliteration by background resemblance, object resemblance, mimicry between unrelated species. With each of these categories associated data are presented, and the relative numbers of deceptive species estimated. Deception is a single phenomenon with many phases, and is important ecologically in proportion to the number of species exhibiting it in a given association, for it slackens the pressure of competition and permits an 'overpacking' of that area in the same way as does the division of the area into niches, or the occupation of particular niches by different species at different times.

143. DISTRIBUTION OF LAND VERTEBRATES AMONG ISLANDS OF EASTERN LAKE MICHIGAN. Robert T. Hatt, Cranbrook Institute of Science. (Lantern; 15 min.)

Distribution of land vertebrates in the islands extending from the Manitowish to the Beaver archipelago may be explained for every species on the basis of present geographical conditions. Amphibia, reptiles, and birds are present or absent on any particular island chiefly on the basis of suitable habitat. Among mammals distribution is determined chiefly on the basis of ability of the animals to get across the water barriers by swimming, by boats, or on the ice. Newly arrived migrants on the Michigan mainland, such as the cottontail, spermophile, prairie deer mouse, and the northern white footed mouse, do not occur on the islands.

144. THE GEOGRAPHICAL DISTRIBUTION OF DIGENETIC TREMATODES OF FISHES OF THE TROPICAL AMERICAN PACIFIC. Harold W. Manter, University of Nebraska. (10 min.)

Digenetic trematodes of fishes of the tropical American Pacific are markedly similar to those of the tropical American Atlantic. Of 82 species collected in the Pacific, 23 are identical in the two oceans, 7 species are almost identical, and 5 additional species belong to amphi-American endemic genera. Thus, 35 species (over 41%) show more or less strong Atlantic relationships. Similarity to other regions is relatively slight. Hosts involved are almost always related but different species. The tendency toward amphi-American endemic species of trematodes is more marked than in the case of the hosts or of free-living groups where there are few identical species in the two oceans but rather numerous amphi-American endemic genera.

Trematodes from the Galapagos Islands are most similar (by far) to the West Indian and Gulf of Mexico region, next most similar to the Pacific Mexican coast, and show much less similarity to the trematode fauna of the Pacific South American coast.

Two possible explanations are suggested: (1) the inter-oceanic transfer of trematodes or trematode eggs by fish-eating birds, and (2) the influence of a former continuity between the two oceans. The latter view is favored by the author.

145. STUDIES ON THE MORPHOLOGY AND LIFE HISTORY OF SPELOTREMA NICOLLI (TREMATODA: MICROPHALLIDAE). R. M. Cable and A. V. Hunninen, Purdue University, Oklahoma City University and Marine Biological Laboratory. (Lantern; 15 min.)

Metacercariae occurring in the blue crab, *Callinectes sapidus*, were fed to six young herring gulls which were killed and examined at varying intervals following

experimental feedings. Each bird yielded a large number of adult *Spelotrema nicolli* while six controls were negative for this species. Since very young metacercariae indicated clearly that the cercaria would be of the Ubiquita type, an extensive search was made for such a larva in mollusks occurring where infected crabs were plentiful. After many unsuccessful attempts, a cercaria of the type sought was found in *Bittium alternatum*. It was found to penetrate the crab by way of the gills and pass through the circulatory system to strands of tissue where encystment occurred. The morphology of various stages in the life history of *S. nicolli* and the process of egg formation in the adult fluke have been studied in detail. The excretory formula of the cercari cercaria is $2[(1+1)+(1+1)]$, that of the metacercaria and adult $2[(2+2)+(2+2)]$.

146. THE ECOLOGY OF THE OPALINID PARASITES OF AMPHIBIA. Frank O. Hazard, Ohio State University and Wilmington College. (Introduced by R. C. Osburn.) (Lantern; 7 min.)

Among the parasites most frequently present in the rectum of frogs and toads are the ciliates of the family Opalinidae. A study has been made of the interrelationships between these ciliates and other organisms present in the same environment. The trematode worm, *Diplodiscus temperatus*, infrequently present, feeds upon them, and a flagellate, *Trichomonas augusta*, oftentimes seems by its numbers to crowd them out. A species of *Endamoeba* is sometimes found within the cytoplasm of certain species of opalinids, presenting a case of true hyperparasitism.

Chemical and physical conditions in the rectum of the host have a marked influence upon the parasites. *Opalina* thrives best within a comparatively narrow range of hydrogen ion concentration. Low oxygen tensions are favorable for this organism. By controlling certain chemical and physical conditions of the medium, *Opalina virguloidea magninucleata* has been cultivated in vitro (in artificial media) for a period of 8 months.

The diet of their host has a marked influence upon the protozoa of the rectum. A carbohydrate diet fed to frog tadpoles stimulates cell division in *Trichomonas* and *Nyctotherus*, and seems to stimulate nuclear division in *Opalina*.

At the time of their host's natural breeding season, the multiplication of opalinids is very greatly increased. Injections of pituitary hormones at other seasons of the year not only stimulate the host's sexual development, but usually also induce a rapid reproduction of the opalinid parasites.

147. STUDIES ON THE DEVELOPMENT OF THE EGGS AND NAUPLII OF A PHYLLOPOD BELONGING TO THE GENUS CYZICUS (ESTHERIA). N. T. Mattox, Miami University. (Lantern; 12 min.)

The eggs of the phyllopod, *Cyzicus* (*Estheria*) sp., which were collected at Oxford, Ohio this past summer, have been subjected to various experiments. The eggs have been in water, in an aquarium, since the death of the adults. Some eggs have been placed directly into fresh fountain water, others have been thoroughly dried over periods varying from one day to several weeks. Some of the dried eggs have been subjected to a temperature of -3°C . for several days and others held at 40°C . In all cases the eggs hatched and produced normal, active nauplii within 3 to 5 days. In several cases it has been noted that a second brood

of nauplii hatch from 3 to 8 days after the first hatching of the eggs. At the time of this writing attempts to raise adults have been unsuccessful. Sixteen days, far short of maturity, is the longest period any have lived.

The nauplii and several of the succeeding instars are without first antennae. This appendage makes its first appearance after about the sixth instar.

148. THE CHANGES IN WATER CONTENT DURING DEVELOPMENT OF THE BEETLE, *PASSALUS CORNUTUS* FABRICIUS. I. E. Gray, Duke University. (Lantern; 15 min.)

During the development of *Passalus* from egg to adult the water content increases approximately 27% and then drops back to its starting point. The moisture content of newly laid eggs averages about 67%. After remaining relatively constant for about 4 days the water content increases steadily until, at the time of hatching on the sixteenth day, it is about 88%. Throughout the three larval instars and the pre-pupal stage it remains practically constant, but increases slightly during the pupal stage. The brightly colored, newly hatched adults contain approximately 92% water. The slow process of sclerotization, often requiring several months, is accompanied by a change in color and a gradual drop in water content until, in older, black adults, a level of about 68%, approximately that of newly laid eggs, is reached.

149. THE ANATOMY OF *FERRISSIA TARDA*. C. Clayton Hoff, University of Illinois. (Introduced by H. J. Van Cleave.) (Lantern; 10 min.)

This detailed study of the anatomy of the Ancyloid snail, *Ferrissia tarda*, is to serve as a basis for study of other species of the same family with the aim of furnishing material which may show phylogenetic relationships based at least partially upon internal characters rather than wholly upon the characters of the radula and shell.

All the systems are described in detail. An important correction is made in the description of the position of attachment of the flagellum duct of the male reproductive system. Previously, this has been described in the genus *Ferrissia* as entering the penis near the entrance of the vas deferens. In *F. tarda*, the flagellum duct enters the praeputium near its attachment to the penis sheath. This condition is similar to the condition in *Pseudoancyclus fluviatilis* of Europe except that the praeputium of the latter is greatly reduced.

A comparison of *F. tarda*, which is a sinistral Ancyloid, with the sinistral *Pseudoancyclus fluviatilis* and the dextral *Ancyclus lacustris* of Europe shows that the American form is more nearly like *A. lacustris* than *P. fluviatilis* in the number of transverse rows of teeth on the radular ribbon, the position and size of the radular sac, and the number of intestinal ceca. A comparison of the genital systems of these three species cannot be given at this time because of a lack of information regarding the system in *A. lacustris*.

150. A PRE-LINNEAN SYSTEM OF BINOMINAL NOMENCLATURE AS DEVELOPED BY THE TUPI INDIANS. G. W. D. Hamlett. (Lantern; 5 min.)

The Indian tribes known collectively as the Tupi or Tupi-Guarini inhabited Brazil from the mouth of the Amazon to the Rio de la Plata and inland as far as

Paraguay. When the Portuguese arrived early in the 16th Century, they found these Indians using a comprehensive and exact double-name system for animals, and apparently for plants also. The first element of the name was a substantive which could be used separately to indicate a group of related animals, or where it was unnecessary to indicate the exact species. The second element was an adjective which modified the substantive. Taken together, the two referred to a single, definite species of animal. The comparison of this with our system of genera and species is obvious. Many of the names have unfortunately been lost with the extinction or absorption of the Tupi tribes, but enough have been preserved to indicate the exactness and comprehensiveness of their taxonomic ideas. A few examples will be given to illustrate the Tupi system.

DEMONSTRATION PAPERS

151. FUNCTIONAL RESULTS OF MUSCLE TRANSPOSITION IN THE HIND LIMB OF THE ALBINO RAT. Roger W. Sperry, The University of Chicago. (Introduced by Paul Weiss.)

See abstract no. 36.

152. SPIRAL MOVEMENT, RHYTHMS AND OTHER CHARACTERISTICS OF LEUCOCYTIC BEHAVIOR. A. A. Schaeffer, Temple University.

See abstract no. 18.

153. KODACHROME PHOTOMICROGRAPHS OF COLOR-PRODUCING CELLS OF BERMUDA CORAL REEF FISH. H. B. Goodrich and Marian Hedenburg, Wesleyan University.

A comparison is presented of the color-producing cell complexes of Bermuda fish with especial reference to a group of closely related parrot fish of the family Sparisomidae. There is a striking similarity of cells and cell arrangement in related species. One taxonomic revision, however, is suggested by the comparative histology. The cells involved are melanophores, erythrophores, xanthophores, iridocytes, and some lens-like cells in the surface epithelium. Blue color appears to be produced by pigmented bodies, in contrast to conditions in marine fish where the blue is caused by refraction of light by crystals. Illustrated with Kodachrome photomicrographs of fresh tissues.

Cytology and Embryology

154. CHROMOSOME DIVISIONS IN THE NURSE CELLS OF THE OVARY OF *DROSOPHILA MELANOGASTER*. T. S. Painter and Elizabeth C. Reindorp, University of Texas.

See abstract no. 126.

155. MORPHOLOGICAL ABNORMALITIES OF *DROSOPHILA MELANOGASTER* MUTANTS ASSOCIATED WITH INFERTILITY. C. P. Oliver and M. Green, University of Minnesota.

The sex-linked recessive mutant spectacle eye is an allele to lozenge. Homozygous spectacle females are infertile. Approximately 80% of homozygous females produce no offspring; those which breed produce an average of six offspring.

Glossy eye is a recessive allele of lozenge, but dominant to spectacle. Homozygous glossy females breed more frequently than spectacle females. Approximately one-half of the glossy females are sterile; and the fertility of those which breed is similar to that of spectacle. In a majority of the sterile cultures, either spectacle or glossy, eggs can be observed; and in the breeding, infertile cultures, many more eggs are produced than develop. Males of both types are fertile. The compound spectacle-glossy females have good fertility. Only about one-quarter of the cultures are sterile; and those which breed produce large numbers of offspring. Homozygous spectacle and homozygous glossy females have similar morphologies of their reproductive tracts. The external genitalia are of normal type; the tubular receptacle is present; and ova are present and abundant in the ovaries and oviducts. However, the spermatheca and parsovaria are absent. The absent structures likely affect the fertility of the females, but some other change is indicated by the fact that the fertile compound, glossy-spectacle, lacks the same structures. Preparations of the genitalia and the technique are to be shown.

156. DIFFERENTIATION OF CHICK EMBRYO THYROIDS IN VITRO. Esther Carpenter, Smith College.

The thyroids of chick embryos incubated for 8 days show no indication of the presence of either colloid droplets or follicles. Thyroids isolated at this age and grown in a mixture of adult fowl plasma and extract of chick embryos of 11 or more days incubation develop colloid and distinct follicles within a week.

157. THE MORPHOLOGY OF LIMBS DEVELOPED FROM HOMO- AND HETEROPLASTIC GRAFTS BETWEEN BIRD EMBRYOS. Herbert L. Eastlick, University of Missouri.

Limb buds have been transplanted successfully in all possible combinations between 2- to 3-day duck, turkey, guinea fowl and chick embryos; also chukar partridge and quail limb buds have been grafted to chick hosts. The grafts, which appear to undergo normal morphogenesis, seem to possess characteristics of the donors. That is, a duck limb bud transplanted to a chick, turkey or guinea fowl embryo develops a typical web foot while a chick limb primordium transplanted to a duck develops into a leg possessing the characteristic appearance of the chick. Moreover, limb buds of White Silkie Bantams (a breed lacking hooklets on the barbules so that the feathers have a plume-like appearance) transplanted to White Leghorn or Barred Plymouth Rock embryos developed feathers which are wholly or in part of donor type. The graft and its feathers seem to grow at the donor rate. During the first few weeks after hatching a duck limb attached to the body wall of a chick grows at approximately twice the rate of the host's own leg in accordance with the faster growth rate inherent to the duck. Chukar partridge and guinea fowl grafts after hatching grow but little as they soon become necrotic and are resorbed.

Limb buds severed from Mallard ducks, Bronze turkeys, etc. in such a way as to exclude neural crest cells develop non-pigmented plumage when placed into the intra-embryonic coelom of pigmented hosts. Similar limb buds attached to the lateral body wall of pigmented hosts always bear colored down since the host melanophores migrate into the grafts.

158. THE FETAL MEMBRANES OF INSECTIVORA. H. W. Mossman, Department of Anatomy, University of Wisconsin.

At least one species of American mole (*Scalopus aquaticus*) possesses a non-deciduate, villous, epitheliochorial placenta. This is the first insectivore shown to have a primitive type of placentation. Its significance in our interpretation of the phylogeny of mammals and of the placenta is considerable. No longer can it be said that the most primitive placental mammals, the Insectivora, all have a specialized type of placentation. Neither is there reason to continue to assume that epitheliochorial placentation is not primitive, nor that placental characters have no phylogenetic value because Insectivora have a specialized type of placentation. The facts now available show that almost all of the fundamental placental types are exhibited within this one order. This is what one should logically expect of such an ancient and widely divergent group. The Talpidae possess in *Scalopus* the most primitive type of placentation in the order. The European mole (*Talpa*) has a deciduate, endotheliochorial placenta, but in other respects its membranes are practically identical with those of *Scalopus*. The placentation of the European shrew (*Sorex*) has been said to be hemochorial, but examination of American *Soricidae* (*Sorex*, *Blarina*, *Cryptotis*) casts considerable doubt on this. According to Sansom, the Indian musk shrew (*Crocidura*) is at least partly hemochorial. The tree shrew (*Tupaia*) is endotheliochorial. The hedgehog (*Erinaceidae*), the Cape gold mole (*Chrysochloridae*), the African water shrew (*Potomogalidae*), and *Centetes*, *Hemicentetes*, and *Ericulus* (*Tenrecidae*) are all hemochorial. Every opportunity should be taken to obtain and study the fetal membranes of insectivores, especially of rare forms.

159. THE FETAL MEMBRANES OF DIPODOMYS (KANGAROO RAT) AND THE PHYLOGENETIC RELATIONSHIPS WITHIN THE GEOMOIDEA AS INDICATED BY THESE STRUCTURES. Paul E. Nielson, Department of Anatomy, University of Wisconsin. (Introduced by H. W. Mossman.)

Information is now available on the fetal membranes of the following Geomoidae: family Geomyidae, genera-*Geomys* and *Thomomys*; family Heteromyidae, genera-*Dipodomys* (including *Perodipus*), *Microdipodops* and *Perognathus*. The fetal membranes indicate a closer relationship between the Geomyidae and *Dipodomys* and *Microdipodops* than between the latter two and *Perognathus*. Although the *Perognathus* material is incomplete, that which is available strongly suggests this genus is the most primitive of the group yet studied.

Implantation in *Dipodomys* is incompletely interstitial and antimesometrial with the inner cell mass on the mesometrial side of the blastocyst. Inversion of germ layers starts before the embryonic disc is formed, no chorio-vitelline placenta exists, and the bilaminar yolk-sac wall has completely disintegrated by the limb-bud stage. Amniogenesis is by a vestigial folding process resulting in the formation of an epamniotic cavity sealed off from the uterine lumen by a prominent trager. The inverted yolk-sac placenta lies against the decidua capsularis until the latter disappears near term, after which the yolk-sac endoderm contacts the uterine mucosa. Near the allantoic placental disc simple yolk-sac villi are present.

No allantoic vesicle is formed. The discoidal, mesometrially situated allantoic placenta has its edges curved over the fetal surface as in *Geomys*. It is labyrinthine hemochorial in type, probably with hemoendothelial areas. The allantoic placentae of *Microdipodops* and *Perognathus* are somewhat similar.

160. HISTOLOGICAL AND CYTOLOGICAL PREPARATIONS FROM AMPHIUMA. Francis H. Wilson, Tulane University.

Not only are the erythrocytes of *Amphiuma* visible to the unaided eye, but most cells are unusually large. Histological and cytological details which require oil immersion or high power in other animals are easily visible under much lower magnification.

Microscopic preparations of selected organs will be exhibited and others will be available for examination.

161. STUDIES ON THE INFILTRATION OF MICROLOGICAL EMBEDDING MEDIA. Robert T. Hance, Duquesne University.
See abstract no. 138.

162. MICROSCOPICAL CHARACTERISTICS OF SOME EMBEDDING MEDIA. Robert T. Hance, Duquesne University.
See abstract no. 139.

163. REST, ACTIVITY AND REPAIR IN CORTICAL CELLS OF THE PIGEON ADRENAL. Richard A. Miller and Oscar Riddle, Station for Experimental Evolution, Carnegie Institution.

This demonstration considers the structural changes which occur in cortical cells of the adrenal glands of pigeons subjected to various experimental procedures. Hypophysectomized young (1.9 months old) pigeons and other normal unoperated birds of the same age were injected with a variety of anterior pituitary preparations. Uninjected normal and hypophysectomized animals served as controls in these tests.

Following hypophysectomy we find not only the expected decrease of interrenal tissue (probably progressive up to 20 to 30 days) but a diminution and disappearance of mitochondria within the cells, a contraction of the Golgi apparatus, together with a persistence and relative increase of the lipid droplets which come to constitute more than half the cell volume of the cortical cells.

Following the injection of an extract of whole anterior pituitary glands, or of an adrenotropic fraction of such glands, the direction of all the above noted structural changes is reversed. Thus, the weight of the adrenal is maintained in the hypophysectomized and increased in the normal animals; the mitochondria proliferate markedly; the Golgi apparatus undergoes great hypertrophy; the lipid droplets decrease in number and almost completely disappear in the highly stimulated glands.

The cytological criteria of rest and activity observed in the bird adrenal are applied to the problem of the normal hormonal mechanism for functional maintenance of the adrenal cortex.

164. EFFECTS OF GONADOTROPIC AND SEX HORMONES ON THE UROGENITAL SYSTEMS OF JUVENILE DIAMOND-BACK TERRAPINS. Paul L. Risley, State University of Iowa.

Recently hatched diamond-back terrapins (*Malaclemmys centrata*) were injected with sheep pituitary extract, testosterone propionate, and estradiol dipropionate in order to determine their effects upon growth and differentiation of the reproductive systems of both sexes. Most effects observed are somewhat similar to those obtained in young alligators by Forbes. Estradiol dipropionate stimulates in males development of the rudimentary ovarian cortex of the testis as well as remnants of the oviducts, but reduction in testis size occurs. In females, ovarian cortex and oviducts enlarge. Testosterone propionate in females produces decreased ovarian size and regression of cortex, but has little effect upon ovarian medulla; oviducts respond by great enlargement, as does the cloaca and glans clitoridis. In males, testes increase in size and seminiferous tubules tend to open and increase in diameter, while the ovarian cortex is reduced to a minimum. Rudimentary oviducts of the males are likewise stimulated slightly, and the glans penis is greatly enlarged. Gonadotropic hormones of the sheep pituitary cause enlargements of both testis and ovary, and certain effects are also seen in secondary sex organs. In females, the oviducts respond by enlargement in some cases, but not in others; in males, the rudiments disappear completely. Numerous effects are noted in the cloacal and duct systems, the details of which will be presented by means of microscopic preparations and photomicrographs.

165. CORRELATIVE CYCLICAL CHANGES IN THE HYPOPHYSIS AND GONADS OF *NECTURUS MACULOSUS* (RAFINESQUE). I. THE MALE. Henry W. Aplington, Cornell University. (Introduced by Howard B. Adelmann.)

The hypophysis of *Necturus* exhibits no real demarcation between pars juxta-neuralis and pars distalis, and it is convenient to subdivide the pars buccalis on the basis of the distribution of cell types into basophilic bed, transitional zone and pars anterior.

In each region one or more cell types predominate: in the basophilic bed, non-granular basophiles with floeculent blue cytoplasm (Masson's stain); in the transitional zone, non-granular acidophiles stained pink to red; in the pars anterior, cells of type 1 (acidophiles whose cytoplasmic granules are bright carmine), cells of type 2 (basophiles with homogeneous purple cytoplasm), and cells of type 3 whose basophilic cytoplasm contains large, red staining granules. Small undifferentiated cells occur uniformly throughout the gland.

Based on monthly cell counts the cyclical behavior of these cell types can be correlated with the cycle of the testis. Type 1 cells increase during spring and early summer, degranulate during the breeding season and return to their winter level during December. Cells of type 3 have a special period of increase from April to June during the growth of the testis and regress from late June to September. Type 2 cells behave reciprocally. The trend of the non-granular basophiles and acidophiles is downward during early spring and summer, upward in late summer and fall.

The conception of pars intermedia, the relations of the cells to one another and the cytology of the hypophysis are related to existing work.

166. EFFECT OF SEX HORMONES ON THE BREEDING PLUMAGE OF WEAVER FINCHES.

Emil Witschi and Paul D. Altland, State University of Iowa.

It was reported in earlier communications that the breeding plumage of male weaver finches develops in response to the release of relatively high amounts of the so-called luteinizing hormone (LH) of the hypophysis. Various estrogenic sex hormones were found to inhibit the assumption of breeding plumage. In a new series of experiments it was established that testosterone as well as androsterone also prevent males and male castrates from assuming the breeding plumage, while at the same time they cause the bills to become darkly pigmented. Obviously the suppression of the breeding plumage is not a direct effect but the injected androgens decrease the output of LH by the hypophysis. Simultaneous injections of LH and of androgenic hormones result in black bills and breeding plumages in males as well as in castrates and females. Injections of androgens, however, do not produce a typical hen plumage. They have a mobilizing effect on the stores of carotinoid pigments (mainly in the liver); as a consequence the whole plumage assumes a bright yellow or orange tint. Injections of androgens, even in small amounts, produce a slow but complete molt of normal appearance.

167. BIOCHEMICAL ASPECTS OF THE DIFFERENTIATION OF THE FEMALE HONEYBEE

(*Apis mellifera* L.). R. M. Melampy, E. R. Willis and R. D. Olsan, U. S. Department of Agriculture and Department of Zoology, Louisiana State University.

The demonstration consists of tables and graphs illustrating the chemical changes associated with the differentiation of the queen and worker castes of the female honeybee (*Apis mellifera* L.).

168. APPARATUS FOR RECORDING CYCLICAL ACTIVITY IN THE RAT. John H. Welsh and Leon Levinson, Harvard University.

A modification of the Hemmingsen and Krarup activity recorder will be shown. This consists of a running wheel and cyclometer, such as used by Richter and others, plus a ratchet mechanism operating a writing lever which gives a continuous record of the number of revolutions of the running wheel per unit of time.

The apparatus is particularly well adapted for recording 24-hour cycles in activity which persist in constant darkness, and for determining the length of the oestrous cycle and the peak of oestrus with relatively little disturbance to the animal.

Records of persisting 24-hour cycles and oestrous cycles in the running activity of the rat will also be shown.

169. A COMPARATIVE STUDY OF THE ELECTRICAL RESPONSES FROM THE COMPOUND EYE. Frederick Crescitelli and Theodore L. Jahn, State University of Iowa.

See abstract no. 66.

170. A DIURNAL RHYTHM IN THE ELECTRICAL RESPONSES FROM THE COMPOUND EYE OF CERTAIN BEETLES. Theodore L. Jahn and Frederick Crescitelli, State University of Iowa.

See abstract no. 67.

171. THE EXPERIMENTAL PRODUCTION OF MELANIN PIGMENT ON THE VENTRAL SURFACE OF THE SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*). Clinton M. Osborn, Ohio State University.

See abstract no. 58.

172. A STUDY OF THE DURATION OF THE INSTARS OF *DAPHNIA MAGNA* WHEN REARED AT A CONSTANT TEMPERATURE. J. C. Jenkins and Bertil G. Anderson, Western Reserve University.

Observations regarding the duration of instars in hours were made on 159 *Daphnia magna* females individually reared for their entire life span at a constant temperature of 25°C. The data were segregated on the basis of pre-adult instars which varied from four to six. The time between the release of the egg and the first ecdysis of the young averaged 69 hours. The instar following (designated as the second instar) averaged 23 hours. Except in those animals which had six pre-adult instars each succeeding pre-adult increased in duration. The length of the last pre-adult regardless of the number preceding varied less than that of the other pre-adult instars, being approximately 32 hours. The first adult instar varied little and was half again as long as the final pre-adult. The durations of the adult instars increase with each instar until the seventh after which they remain relatively constant.

173. THE SUBSTITUTE FOR A VASCULAR TRANSPORT SYSTEM IN *ASTERIAS*. R. A. Budington, Oberlin College.

A group of photographs of external and internal epithelial surfaces of *Asterias forbesii* on which are indicated the fluid currents propelled by cilia. The location and direction of current-tracts are as constant as those of arteries and veins in animals with a conventional vascular system.

174. POPULATION FLUCTUATIONS IN *PARAMECIUM* CULTURES. Edgar P. Jones, University of Akron.

See abstract no. 98.

175. CYTOLYZING ACTION OF *TARANTULA* VENOM. Edgar P. Jones, University of Akron.

See abstract no. 99.

176. ISLET CELL TYPES AND THEIR TOPOGRAPHICAL RELATIONSHIP TO THE DUCT EPITHELIUM AND EXOCRINE TISSUE IN THE PANCREAS OF CERTAIN ELASMOBRANCHS. Thurlo B. Thomas, Phillips Exeter Academy.

A, B and D types of granular islet cells have been identified in the pancreas of six species of elasmobranch fishes following Helly's fixation and Heidenhain's azocarmine stain.

Using as a criterion the relationship of the islet tissue to the duct system, the animals studied may be divided into three groups. (1) The most primitive

condition in which the islet cells form a second epithelial layer around the duct cells, (2) An intermediate condition in which some islet cells are found in their primitive position, but most of them occur as solid cords of cells contiguous to the ducts, (3) The most advanced condition in which some cells occur as in each of the first two groups, but the majority appear in the form of mammalian-like islands frequently separated from the duct system.

In all elasmobranchs examined the exocrine tissue is frequently invaded by one or more islet cells. These may be crowded between the bases of the acinar cells, or they may assume a position in the acinus typical of an exocrine cell.

177. A PRONEPHRIC KIDNEY COMBINED WITH A SIMPLE MESONEPHROS IN A TELEOST FISH, *MACROSTOMA (LAMPANYCTUS) WARMINGI* LÜTKEN. Bradford B. Owen, Harvard University. (Introduced by Alden B. Dawson.)

A graphic reconstruction has been made showing the plan of the entire kidney. The pronephric portion consists of two large, closely-approximated glomeruli lying on the surface of the dorsal aorta near the level of the last gill arch, and of two large tubules associated with them by means of the usual Bowman's capsule. These tubules possess a short ciliated neck, followed by a brush-border epithelium of the proximal convolute type. They extend caudally to connect with the bladder. This pronephros is comparable to that which forms the entire kidney of *Cyclothone*.

In *Macrostoma*, however, there are also mesonephric tubules. These are few in number (about twenty-five on each side), and arise in association with smaller glomeruli occurring at regular intervals, usually in groups of two to four, along the dorsal aorta. They drain into the paired pronephric tubules, which thus become the mesonephric ducts, although preserving a secretory epithelium over much of their length. Before reaching the bladder, this epithelium loses its brush border and resembles that of typical collecting ducts. A single, smaller collecting duct extends posteriorly from the bladder to receive mesonephric tubules from the more caudal groups of glomeruli.

Each mesonephric tubule consists of a capsule of Bowman, a short, ciliated neck region, and a proximal convoluted segment. The latter shows two regions, distinguished by a fairly abrupt transition between two types of brush-border cells.

178. STRUCTURE OF THE SKIN OF THE SOUTH AMERICAN FROG, *LEPTODACTYLUS PENTADACTYLUS*. Elbert C. Cole, Williams College.

The skin of this frog is smooth and thin, varying from $\frac{1}{4}$ mm. on the throat to $\frac{1}{8}$ mm. on the dorsal surface of the thigh. Scattered glands, exclusively of mucous type, are present. The dorsal epidermis is four cells deep and has an indistinct stratum germinativum. The ventral epidermis is six cells deep and its stratum germinativum consists of distinct columnar cells.

A loose meshwork of collagenous fibers forms the upper layer of the dermis, in which are glands, capillaries, and branching melanophores. Guanophores are scattered and infrequent.

In the deeper dermis is a thick layer of compact collagenous fibers, lying in wavy bundles parallel to the surface of the skin. At intervals, vertical bundles divide this layer into segments. Each segment is rounded, and capped by a dense

mass of extremely fine tangled fibrils and minute granules, both of which are strongly basophilic. This segmentation is absent in the dorsal skin of the thighs. The dense dermal layer represents the inner boundary of the skin, except in the throat, where a loose connective tissue layer lies between it and the musculature of the throat.

Points of contrast between the skin of this frog and that of *Rana pipiens* are: (1) the absence of serous glands, (2) the small number of mucous glands, (3) the great thickness of the dense collagenous dermal layer, (4) the division of this layer into segments, and (5) the basophilic nature of the segment caps.

179. MOLTING AND FORMATION OF THE EXOSKELETON IN THE BRINE SHRIMP, *ARTEMIA GRACILIS* VERRILL. John H. Lochhead, Harvard University. (Introduced by A. C. Redfield.)

When kept at room temperature the early nauplii of the brine shrimp molt about every 24 hours, the time increasing later to about 48 hours. At the onset of sexual maturity there is a sudden jump to a regularly recurring cycle of about $4\frac{1}{2}$ days. This cycle continues throughout the rest of the life of the animal and it is closely associated with the reproductive processes.

The exoskeleton is entirely a product of the hypodermal cells. In most parts it is less than 2μ thick and consists of a single layer of chitin and 'cuticle' mixed together. In a few places it is thicker and is clearly divided into an inner chitinous layer and an outer cuticular layer. Secretion of a new exoskeleton is preceded by a separation of the old exoskeleton from the hypodermis. The inner layers of the old skeleton are not digested or absorbed. When two layers of the skeleton are present the outer, cuticular layer is secreted first; secretion of the inner, chitinous layer is often delayed until after the occurrence of the molt. Formation of most of the new skeleton begins only 1 day before a molt, but secretion of the thicker parts begins rather earlier.

This whole molting process is simpler than any previously described. In particular there are no accessory glands such as have been described for insects and for higher Crustacea.

180. ORIGIN AND FATE OF BLOOD CELLS IN THE BRINE SHRIMP, *ARTEMIA GRACILIS* VERRILL. Margit Szabó Lochhead, Harvard University. (Introduced by A. C. Redfield.)

The site of blood cell formation in *Artemia* has been found in twenty-two special organs, one placed near the base of each trunk limb. Each organ consists of a mass or nodule of round cells enclosed within a very thin membrane. Most of the cells have a large round nucleus and relatively little cytoplasm. They reproduce by mitotic division. Near the periphery and outside of the nodules, cells are found with smaller nuclei and more cytoplasm, representing all stages between the younger cells and the older amoebocytes found free in the blood. These amoebocytes are the only type of blood cell present. Nearly always they carry inclusions in the cytoplasm. Among those free in the blood mitoses are of extremely rare occurrence.

Considerable numbers of the amoebocytes are often taken up and digested by large, stationary phagocytic cells which are present in various parts of the body.

181. THE VENOM GLANDS OF THE BLACK WIDOW SPIDER. Albert M. Reese, West Virginia University.

The histological of these glands seems uncertain and will be described in a future paper.

182. DISTRIBUTION AND COLOR OF *HELIX HORTENSIS* ABOUT GASPÉ BAY, QUEBEC. Stanley C. Ball, Yale University.

The European land snail, *Helix hortensis*, having invaded or been introduced into northeastern America perhaps in pre-Columbian time, is now distributed from Massachusetts to Anticosti. About Gaspé Bay this species has been found only along the coast and a few miles inland, occupying stations from the shoreward margin of vegetation through cultivated land to the forested summits of 1200 foot elevations.

In the two chief biotopes, grassland and mixed forest, these snails present marked differences in pigmentation, both in body and shell. With few exceptions the grassland type develops little or no body pigment, while its shell is light yellow with more or less distinct but narrow reddish bands. On the other hand, the forest type develops much melanin over the body, with darker and wider shell bands that tend to fuse. The extreme is a uniform bay shell whose appearance is deepened to seal brown by the slaty black body within.

Bandless shells occur in both types; if in grassland the living animal appears yellow, while the dark body of the forest snail produces a greenish effect through the translucent yellow shell. Intermediate colors and widths of bands, seen over varying depths of body color, give a whole series of color variations in snails inhabiting different facies of the two biotopes.

Experimental field planting and laboratory terraria are being studied to determine the importance of genetic and ecologic factors.

PAPERS READ BY TITLE

183. ON THE EFFECTS OF EXTREME COLD AND HIGH VACUUM ON THE DEVELOPMENT OF *ARTEMIA* CYSTS. D. M. Whitaker, Stanford University.

Air dried cysts of *Artemia* from the vicinity of San Francisco Bay were suddenly subjected to the temperature of liquid air ($-190^{\circ}\text{C}.$), and were maintained at this temperature for 7 and 24 hours. Other cysts were maintained in high vacuum (approx. 10^{-6} mm. Hg) at room temperature for periods up to 6 months. Neither of these treatments impaired either the percentage or rate of excystment.

184. MOSAIC DEVELOPMENT IN THE ANNELID EGG. D. P. Costello and H. M. Costello, University of North Carolina.

E. B. Wilson in 1904 demonstrated that the development of the annelid *Lanice* as well as of the molluscs *Dentalium* and *Patella* could be regarded as a mosaic-work of self-differentiating cells. Preliminary experiments, performed during the summer of 1939 on the development of isolated blastomeres of the eggs of *Nereis limbata*, contribute further to this problem. The vitelline membranes of the eggs of *Nereis* were removed shortly after insemination by a method previously de-

scribed. The blastomeres were separated by hand with micro-needles at the two-, four-, and eight-cell stage, and their development followed in isolation culture. The CD (posterior) cell develops (in the best of 140 operated cases) into a partial trochophore with prototrochal cilia, two oil drops, prototrochal pigment, anal pigment, and one (rarely two) eyespots. The development of the AB (anterior) cell contrasts sharply with that of the posterior. These embryos (140 isolations) likewise produce a prototroch, and very rarely a small patch of prototrochal pigment, but none developed a definite eye, although there was one doubtful case.

If the blastomeres are separated at the four-cell stage, all four develop a partial band of prototrochal cilia. Prototrochal pigment and an eyespot were found only in C or D quarter embryos. At the eight-cell stage any isolated micromere may develop prototrochal cilia and rarely certain micromeres develop a small amount of prototrochal pigment or an eyespot. The isolated macromeres never become ciliated, and develop neither pigment nor eyespots. This indicates that the prototrochal material is segregated into the micromere region before the third cleavage, as would be expected from the cell-lineage of this form.

185. STUDIES OF FERTILIZATION. I. THE FERTILIZABILITY OF NUCLEATED AND NON-NUCLEATED FRAGMENTS OF CENTRIFUGED NEREIS EGGS. D. P. Costello, University of North Carolina.

When the unfertilized eggs of *Nereis limbata* are centrifuged at 66,000 times gravity for 60 to 90 minutes, the interior protoplasm becomes stratified and the eggs are elongated into a dumb-bell form. Pairs of egg fragments were obtained by cutting the dumb-bells immediately after removal from the centrifuge, by hand, with micro-needles. Four types of cuts were made: 'upper,' across the neck of the dumb-bell; 'lower,' across the lower edge of the yolk zone; 'intermediate,' through the yolk zone; and through the hyaline zone between the germinal vesicle and the oil cap. The germinal vesicle is contained in the centripetal fragments of upper, lower and intermediate cut eggs and in the centrifugal fragments of the eggs cut between the oil cap and the germinal vesicle.

Upon insemination, either fragment of a pair, or both, may be activated. Sperm entrance into both nucleated and non-nucleated fragments was observed. The activated centripetal fragments of upper, lower and intermediate cut eggs usually cleaved, whereas the corresponding centrifugal fragments never cleaved. On the other hand, of the eggs cut above the germinal vesicle, the centrifugal fragments usually cleaved upon insemination, while the centripetal fragments did not.

These experiments demonstrate that, although both fragments of the *Nereis* egg may be activated, the presence of the germinal vesicle is necessary for the completion of fertilization. The egg of *Nereis* is apparently incapable of merogonic development.

186. THE STRUCTURE OF THE POLAR BODY AND EGG CORTEX IN CHAETOPTERUS. Leonard G. Worley, Brooklyn College.

The cortex of both the unfertilized and the fertilized *Chaetopterus* egg is crowded with dictyosomes, except in the area described by Hoadley as the 'ectoplasmic defect.' Both dictyosomes and mitochondria are scattered in the endoplasm. The 'ectoplasmic defect' is also devoid of yolk, mitochondria and other

osmiophilic material. In 55% of a large number of cases examined, polar bodies emerged from the egg lacking in all of these elements. About 25% apparently contained one or two chondriosomes. The condition of the remainder was questionable. Typically lacking as they are in these important cytoplasmic components of the cell, it is not surprising that the polar bodies in *Chaetopterus* fail to divide and the use of the term 'polocytes' for such structures does not seem justified.

187. THE BEHAVIOR OF THE DICTYOSOMES DURING CLEAVAGE AND GASTRULATION IN ARBACIA. Leonard G. Worley, Brooklyn College.

In the fertilized egg of *Arbacia*, the dictyosomes are small, scattered rings; the ectoplasm shows a great concentration of mitochondria. During early cleavage, the dictyosomes increase in size and occupy the central cytoplasm of the blastomeres; the nucleus moves to a position near the outer surface of the cell; the chondriosomes occupy the opposite end. In the early blastula, the nucleus shifts back toward the center of the cell, while the dictyosomes pass around the nucleus to a peripheral position. Here, they unite to form a conspicuous W-shaped reticulum, which frequently fits like a cap on the nucleus. Endoderm cells retain this arrangement during gastrulation with the result that in the cells lining the early archenteron, the Golgi reticulum is adjacent to the lumen; i.e. in the distal cytoplasm of the young gut cells. The typical morphological polarity of the digestive epithelium is established in *Arbacia* cells in the early blastula stage.

188. MICRODISSECTION OF A CILIATED EPITHELIAL CELL. Leonard G. Worley, Brooklyn College.

Ciliated simple columnar epithelial cells (length about 60μ) from the intestine of *Anodonta cataracta* Say were cut or injured at various distances from the distal margin to determine the effect of such operations on the coordinated metachronal action of the cilia. These studies indicate that coordination is immediately altered by injuries to approximately the distal third of the cell; the cilia revert to synchronic action or become completely incoordinate. Cuts made in the proximal two-thirds of the cell provoke no immediate effect other than occasional beat cessation. Similar results have been obtained in *Venus* and *Sphaerium*. It is concluded that the distal third of the cell is responsible for the metachronal activity of cilia. Since this is the region occupied by the ciliary cone, a function of coordination for this cell structure is suggested.

189. ABNORMALITIES PRODUCED IN *TRIBOLIUM CONFUSUM* DUVAL BY EXPOSURE TO A SECRETION OF THEIR OWN MANUFACTURE. Louis Roth and Ruth B. Howland, Washington Square College, New York University.

Adults of the confused flour beetle, *Tribolium confusum*, give off a pungent gas when irritated by external factors such as mechanical stimulation, light or temperature changes. The gas may be collected by passing dry air over a vessel containing stimulated adult beetles and into a trap packed in dry ice and acetone (at -60° or below). At room temperature, the resulting dry ice condensate becomes liquid and is extremely volatile. Its odor suggests that it is one of the quinones. Like the quinones, the secretion undergoes peculiar color changes and

is capable of releasing I from KI. The regions from which the gas is thrown off can be detected by holding a beetle against KI starch paper moistened with acidulated water. Prepupae of *Tribolium confusum* were exposed to gas and subsequently incubated at 25°C. A large proportion of the pupae resulting from these showed melanized areas of varying extent, in legs, antennae and mouth parts. In adults from such pupae part or all of the affected organs were missing. Pupae exposed to the gas gave adults with minor abnormalities of a different character, for example, spread and partly unsclerotized elytra.

190. EFFECTS OF EARLY CENTRIFUGATION ON *DROSOPHILA* EGGS, EMBRYOS, LARVAE, PUPAE AND ADULTS. Ruth B. Howland, Harry G. Albaum and Samuel Kaiser, Washington Square College, New York University and Brooklyn College.

Centrifugation of timed eggs for 30 minutes at 2400 r.p.m. stratifies the egg constituents into four clearly defined zones in addition to the already differentiated poleplasm which though shifting slightly retains its terminal position. Centrifugally, overlying the poleplasm is a homogeneous layer formed by the confluence of the vacuolar fluid surrounding the yolk spheres. Next to this lie the free, heavy yolk-spheres. Grading through the smaller yolk-spheres, this zone borders on a broad finely granular or fibrillar layer in which the egg nuclei lie. Centripetally is found a clear non-granular 'cap.' Giant polar-body complexes with multipolar spindles, asynchronous mitoses of cleavage nuclei, giant cleavage nuclei, and lagging chromosomes are common. In centrifuged preblastoderm stages, the four zones just described occupy the egg center. The preblastodermic nuclei, though lacking cell boundaries, seem bound in the cortical ooplasm, which has now apparently become more viscous. Larvae from centrifuged eggs hatch later than normal larvae. In general, a larger percentage of early eggs hatch after either dorsal or posterior than after ventral or anterior centrifugation. Hatched larvae which do not pupate may appear normal but sluggish, often have tracheal tubes in a snarled mass posterodorsally, or show partial torsion or rotation along the anteroposterior axis. Many unhatched larvae have malformed jaw armatures. In both pupae and adults, torsion of body regions along the anteroposterior axis is common. It is suggested that such displacements are due to lateral masses of centrifuged material which interpose a mechanical block during the normal elongation of the germ band.

191. THE TEMPERATURE-EFFECTIVE PERIOD AT 16°C. FOR THE GENOTYPE vg^{No3}/vg^p OF *D. MELANOGASTER*. Adele I. Cohen and Morris H. Harnly, Washington Square College, New York University.

The phenotype of vg^{No3}/vg^p for total development at 16°C. is large 'strap' for the males and small 'strap' wings for the females and at 25° it is over-sized 'vestigial.' The temperature-effective period at 16° has been determined by transfers, at regular intervals, of developing individuals from 16° to 25°. All larvae transferred between 4 and 7½ days of total development become flies with wings typical both in length and phenotype for flies reared at 25°. Larvae transferred at 8 days have markedly larger wings on both sexes. There is a further increase in wing size at 16° until the females reach 11 days and the males 17 days of development. Beyond these points there is no further increase in wing length or

change in phenotype. The temperature-effective period is between $7\frac{1}{2}$ days and 11 days at 16° for the females and $7\frac{1}{2}$ days and 17 days for the males. Both curves are sigmoid and have approximately the same slope. The difference in wing size between the males and the females is obviously due to the difference in the duration of the effective period. A detailed study of the developing imaginal discs is being made to determine the relation of the temperature-effective period to the development of the disc.

192. THE EFFECT OF DISTILLED WATER AND ICED DISTILLED WATER ON THE VIABILITY OF DEVELOPMENTAL STAGES OF *RANA PAPIENS*. Linden F. Edwards, Ohio State University. (Introduced by Clinton M. Osborn.)

Developmental stages of *Rana pipiens* (yolk-plug, tail-bud and early free-swimming larvae) were reared at room temperature in measured quantities of distilled water, iced distilled water (obtained by melting ice made from samples of the same distilled water) and of tap water, those in the latter serving as controls. Samples of water were renewed daily and specimens were reared in same sized glass containers. To test effect of samples of water on viability equal numbers of specimens were reared in same quantity of water. Each test was terminated when all specimens in any one sample of water had expired. In all tests those reared in distilled water were first to die, survival periods ranging from 3 to 13 days, whereas 72% of specimens reared in iced distilled water and 96% of the controls survived over the same period. No apparent correlation existed between survival period and developmental stage, amount of water or size of population. The results of this study suggest therefore that the developmental stages of *Rana pipiens* which were reared in iced distilled water exhibit more viability than those reared in distilled water.

193. ABNORMALITIES IN FROG EMBRYOS INDUCED BY CENTRIFUGATION. T. W. Torrey and W. R. Breneman, Indiana University.

Eggs of the frog, *Rana pipiens*, were centrifuged at various stages of development and at various speeds. The developmental stage most affected by centrifuging, as reflected by resulting abnormal larvae, was that of the early blastopore. The most effective centrifugal speed was 2400–2500 r.p.m. Numerous embryos with accessory tails and under-developed or defective heads were obtained. The accessory tails possessed a spinal cord, notochord, and muscle somites. The head abnormalities included: (a) total or partial absence of the forebrain and mid-brain and doubling of the hindbrain; (b) cases of total absence of the eyes, complete cyclopia, and partial cyclopia, the latter two featured by the absence of lenses except for one instance of lens regeneration from the iris; (c) absence of the oral suckers, olfactory organs, stomodaeum, and hypophysis; (d) variable duplications of the otoeysts, uncorrelated with doubling of the hindbrain, and one case of their complete elimination; (e) suppression of development of the prechordal mesoderm; (f) suppression and distortion of the pharynx and visceral arches; (g) one case of total absence of the heart and its reduced size in the other cases; and (h) one instance of an accessory pronephros associated with the secondary embryo. The abnormalities have been interpreted in terms of a presumed shift of the 'head organizer' by the action of centrifugal force.

194. ANDROGENETIC HYBRIDIZATION USING *RANA BRACHYCEPHALA* (COPE) AND *RANA PAPIENS* SCHREBER. K. R. Porter, Princeton University.

A successful technique for androgenesis (described in preceding paper) makes possible the association of the nucleus of one species with the cytoplasm of another species. Reciprocal diploid and androgenetic haploid hybrids have been made between *R. pipiens* of southern New Jersey and *R. brachycephala* of Vermont (Stejneger and Barbour, '39) which differ in body proportions, pigment pattern, etc. Careful observations on the external characteristics of the 3-day-old representatives of the eight possible combinations reveals interesting differences of which only one or two can be mentioned here. The diploid controls of the two species are very similar, but *R. brachycephala* differs from *R. pipiens* in having a slightly larger head, slightly different head flexures and a smaller tail-bud. The androgenetic haploid controls of the two species show differences similar to those of the diploid controls. Of the diploid hybrids the combination *R. brachycephala* egg with *R. pipiens* sperm has a distinctly larger head and a smaller abdomen and tail-bud than the reciprocal. They develop through metamorphosis into normal appearing young frogs. Of the androgenetic hybrids, the haploid *R. brachycephala* cytoplasm with *R. pipiens* nucleus has a much larger head, a smaller tail-bud, and a slower development (in its early stages) than its reciprocal. Both types develop for 7 or 8 days into tadpoles of 5 to 6 mm. Differences which are distinctly shown by hybrid diploids are greatly exaggerated in hybrid haploids. It is possible to find the earliest expression of these differences in the gastrula stages. It is suggested that a portion of the responsibility for these results exists in the cytoplasmic organization of the egg.

195. HYBRIDIZATION IN FROGS. John A. Moore, Columbia University. (Introduced by A. W. Pollister.)

Cross fertilizations were carried out among members of the genus *Rana*. Groups of closely related frogs such as *Rana pipiens*, *Rana palustris*, and *Rana sphenoccephala* hybridize well and the offspring have been raised beyond metamorphosis. In crosses between any of the above-mentioned frogs and *Rana clamitans*, *Rana catesbeiana*, or *Rana sylvatica* the hybrids develop to the beginning of gastrulation and die. Death at this early stage also occurs in crosses between *Rana clamitans* or *Rana catesbeiana* and *Rana sylvatica*.

A study is being made of the rate of development of hybrid eggs as evidence on the time at which the sperm exerts its influence. Normal eggs of *Rana palustris* develop more slowly than those of *Rana pipiens*. Hybrids of *Rana pipiens* female and *Rana palustris* male are maternal in rate until the end of gastrulation when they become retarded. The rate of development in the reverse cross, *Rana palustris* female and *Rana pipiens* male, is maternal until the end of gastrulation after which there is a definite acceleration of development. Therefore the sperm influence on rate of development begins at least as early as the end of gastrulation.

196. GLYCOGENETIC ACTIVITY IN EARLY STAGES OF THE DEVELOPING AMPHIBIAN. Martha Revel Barnes, University of Illinois. (Introduced by Waldo Shumway.)

Preliminary studies have been made on the relative amounts of the enzyme, glycogenase, produced or activated during the early development of *Rana pipiens*,

Bufo americanus, and *Amblystoma tigrinum*, from the unfertilized egg through neural fold formation. After acetone treatment, the enzyme was extracted from lots of fifty dried ground eggs and allowed to react on a 0.1% glycogen solution for 6 hours at 37.5°C. It was then analyzed for glucose which was shown to be proportional to the amount of enzyme present.

Before fertilization in the case of the frog, sufficient enzyme is present, in such a preparation, to hydrolyze approximately 1 mg. of glucose. With onset of cleavage, the amount of enzyme decreases more than 50%. During gastrulation, the glycogenase reaches a level 45% higher than that of the unfertilized egg. A slight drop seems to occur during post-gastrulation, but after 35 hours, the production of glycogenase is slow and constant until the appearance of the neural folds when an increase of 79% takes place. Similar results were obtained with the toad and the salamander.

The results are in accordance with the work of Brachet and Boell on respiratory quotients and with the results of Woerdeman on glycogen occurrence. Further studies are in progress.

197. DEVELOPMENTAL RATE AND ALTERNATING TEMPERATURE. J. William Buchanan, Northwestern University.

A. punctatum embryos were subjected for several days to a temperature of 21°, then removed to 13°. Control embryos from the same source were maintained constantly at 13°. Progress of development in the two lots was followed by comparison with the Harrison stages. It was found that those treated with the higher temperature and thus accelerated, slowed down appreciably to a rate less than that of the controls when placed at the control temperature. The decelerated development of the treated embryos tended to bring their stage-time condition to that of the controls.

When embryos are changed from 5° to 21° and returned to the lower temperature, alternating high and low temperature every 6 hours, they develop at approximately the same rate as do controls maintained at the mean temperature, 13°. But if the intervals of temperature alternation are 12 or 24 hours the treated embryos develop appreciably more rapidly than do controls at the mean temperature. The embryos alternated every 24 hours develop somewhat more rapidly than do those changed every 12 hours. Measurements of final length of the embryos show that growth as well as development is influenced by the treatment.

A simplified hypothesis involving the postulation of two substances and two sets of reactions in development explains the facts. But no attempt is made to extend the hypothesis to cover accelerations following inhibition of development by chemical agents, nor to apply the theory to acclimatization in general.

198. THE OXYGEN CONSUMPTION OF THE AMPHIBIAN ORGANIZER. L. G. Barth, Columbia University.

The oxygen consumption of the organizer region of *Amblystoma punctatum* at S10 was compared with that of the presumptive neural plate, the presumptive epidermis and the presumptive ventral lip of the blastopore. The organizer region consumes 22.0 mm.³ of O₂ per hour per 100 mg. dry weight at 19°C. The pre-

sumptive ventral lip on the opposite side of the egg respire at a rate of 12.0. The two regions were carefully cut out so as to contain about the same amount of yolk in the cells. The values for the presumptive neural plate and epidermis were 23.2 and 20.8 respectively. Yolk cells from the vegetal pole respire at a very low value of 1.6 mm.³ of O₂ per hour per 100 mg. dry weight.

The results show that the dorsal lip region respire at a much higher rate than the corresponding region on the opposite side of the egg where the ventral lip will form. The O₂ consumption of the presumptive epidermis and dorsal lip region cannot be accurately compared until the yolk content of the cells of these regions is determined. Since yolk-containing cells respire at a very low rate and since the dorsal lip contains many cells with large amounts of yolk, it is not valid to compare the O₂ uptake of the dorsal lip with the presumptive epidermis which is relatively free of yolk. Although the dorsal lip respire at a rate of 22.0 and the presumptive epidermis at 20.8 the active protoplasm of the dorsal lip must be respiring much more rapidly than that of the presumptive epidermis if corrected for the different yolk contents.

199. NEURAL DIFFERENTIATION WITHOUT ORGANIZER. L. G. Barth, Columbia University.

Explants of the presumptive epidermis and presumptive neural plate of S10-11 of *Amblystoma punctatum* were found under certain conditions to form very well-defined neural tubes. The tissue explanted had not been acted upon by the organizer but was still the roof of the blastocoel. Two of these explants when fused by their anterior ends (presumptive neural plate) form neural tubes at the region of fusion. When the anterior half of an explant is fused with the anterior end of a whole explant a well-defined neural tube differentiates from this anterior end.

Lateral fusion of two explants does not give neural tubes if the anterior and posterior ends fuse with each other. The fusion of the posterior margin of the presumptive epidermis with the presumptive neural plate inhibits the differentiation of the neural plate.

Finally, a single explant can form a neural tube if the lateral edges are brought together to form a tube having an anteroposterior axis. However, if the anterior and posterior edges are brought together a neural tube rarely forms. Again, the fusion of the anterior and posterior ends inhibits neural tube differentiation.

The results are explained by the gradient theory of Child with the neural tube forming at the high end of the gradient in the ectoderm. If this gradient is preserved in explant by proper fusion and healing a neural tube forms. If the gradient is weakened or obliterated by the fusion and healing only epidermis results.

200. EXPERIMENTAL ANALYSIS OF THE DEVELOPMENT OF THE ANURAN OLFACTORY ORGAN. Edgar Zwilling, Columbia University. (Introduced by L. G. Barth.)

It has been ascertained, by transplanting portions of the head ectoderm of various gastrula stages to the flank of older urodele embryos, that the olfactory organ of *Rana pipiens* is determined at a stage prior to the formation of the

neural plate. By implanting portions of the anterior roof of the archenteron into the blastocoels of gastrulae in which the blastopores were round it was found that the olfactory organ may be induced despite the absence of brain. Anterior neural plate implanted into similar hosts failed to give such inductions except in one doubtful case. Anterior neural plate or forebrain of older embryos did not induce olfactory structures when transplanted under the flank ectoderm of neurulae. The inner ectodermal layer of the presumptive olfactory tissue can self-differentiate into olfactory tissue; and can induce a nasal pit in ectoderm which is placed above it. By means of heteroplastic transplants between *Rana pipiens* and *Rana palustris* it was found that the presumptive epidermis of a gastrula with a crescent-shaped blastopore may form an almost perfect chimaera when transplanted to the head region of a very late gastrula. This ectoderm may form olfactory structures in the head region of an early neurula. Ectoderm from the anterior flank region of early neurulae did not form olfactory structures unless some of the inner olfactory ectoderm was present.

201. EXPERIMENTS ON THE 'DETERMINATION' OF THE FIN ECTODERM IN SALAMANDER EMBRYOS. Victor C. Twitty, Stanford University.

The capacity of flank ectoderm to participate in the formation of the dorsal trunk fin was tested by grafting it in substitution of the ectoderm covering the neural tube. The experiments were performed on embryos in 'tail-bud' stages, using three species of *Triturus* and three species of *Amblystoma*.

(1) Hosts of different species exert 'fin-forming stimuli' of different strengths. Thus identical grafts may form fins on hosts of one species, fail to do so on hosts of another. (2) The power of the stimulus declines with age. (3) The decline begins anteriorly. As increasingly older hosts are employed the transplanted ectoderm must be placed further posteriorly to obtain fin formation. (4) The capacity of the ectoderm to respond to the fin stimulus varies markedly in degree with the species. Thus the ectoderm of one species may form a fin, that of another fail to do so, when grafted to identical hosts. (5) Ectodermal responsiveness declines with age, although the change is less marked than one might anticipate. (6) Specific differences in both ectodermal responsiveness and strength of fin stimulus are clearly modifiable by paternal factors, as demonstrated by experiments with hybrid embryos.

The results show clearly that fin formation ensues when two variables (those of stimulation and response) together attain a certain value. A low value for one may be compensated for by raising that of the other. Fin development is thus an expression of relative, rather than critical or absolute, conditions in the activating and reacting tissues involved.

202. EMBRYONIC INDUCTION IN REGENERATING TISSUE OF *RANA PIPENS* AND *RANA CLAMITANS* LARVAE. Henry S. Emerson, Yale University and University of Michigan. (Introduced by W. T. Dempster.)

A total of 336 operations were made transplanting the presumptive medulla, presumptive spinal cord, and presumptive forebrain from neurula stages into the regenerating tail blastema of 3- to 4-cm. tadpoles. Both the histogenesis and the morphogenesis of the grafted tissues were surprisingly normal.

In 11% of the cases small vesicles were formed adjacent to the medulla. These structures are very similar to an early otic vesicle. They have approximately the same position next to the medulla, and the same general shape. They are formed of a single layer of columnar epithelium, similar histologically to young ear vesicle epithelium. In the oldest cases studied the vesicles are similar except that the epithelium is very thin, and in certain cases the vesicles are divided into chambers. There is some evidence that the vesicles are formed by a gradual transformation of a concentration of host mesenchyme cells. The host origin of the vesicles is shown in most instances by clear differences in staining between host and donor tissues, and in cases fixed less than 5 days after operation the grafted embryonic tissue is still loaded with yolk platelets, whereas there are no yolk platelets at all in the vesicles. In the control series of 105 presumptive spinal cord and presumptive forebrain grafts no vesicles of any sort were formed from the blastema tissue.

203. FORE LIMB OPERCULAR PERFORATION IN *R. CATESBEIANA*. O. M. Helff, New York University.

The causative factors associated with fore limb opercular histolysis and perforation formation in *R. catesbeiana* were determined on the basis of the results obtained from the following experiments:

Reciprocal skin transplantations were made between the opercular region and the side or back.

The right fore limb was extirpated in one series and in another to include the adjacent portion of the shoulder girdle which possesses an integumentary covering.

The entire integumentary coverings of the fore limb and adjacent shoulder girdle were removed, the two structures being otherwise undisturbed.

The entire fore limb was implanted beneath back integument.

Cutaneous grafts obtained from the shoulder girdle were transplanted beneath back integument and beneath side and opercular skin grafts previously transplanted to the back.

Atrophying gill tissue was placed beneath back integument and beneath side and opercular skin grafts previously transplanted to the back.

The results of the above experiments lead to the following conclusions: (1) There is no clear-cut evidence that opercular integument is more susceptible to histolysis and subsequent perforation formation than side or back skin. (2) Opercular histolysis is normally largely dependent on factors associated with the integuments of the fore limb and adjacent shoulder girdle. (3) The histolytic effect of atrophying gill tissue is not as pronounced in this species as in the non-neotonic forms previously studied. It is a contributing factor, however, to opercular histolysis, especially when fore limb development is retarded. (4) Limb pressure is quite likely an accessory causative factor.

204. STUDIES ON THE ANURAN RESPIRATORY SYSTEM. I. HISTOLOGICAL CHANGES IN GILL AND LUNG TISSUE DURING GROWTH AND METAMORPHOSIS. C. M. Harrold, Jr., New York University. (Introduced by O. M. Helff.)

A histological study was made of the development, cellular differentiation, and degeneration of the external and internal gills of *Rana sylvatica*. The develop-

ment and cellular differentiation of the lungs were also studied in the same species. The sequence of histological changes in the gills and lung, associated with growth and metamorphosis, was worked out from serial sections of stages extending from the onset of heart beat up to and including the adult frog.

The following facts were determined:

1. Origin of internal gills and of the lungs occurs within the same 24-hour interval.

2. Internal gill filament development precedes appearance of the 'filter apparatus.'

3. Cellular differentiation and presumably function of the internal gill precedes the onset of external gill degeneration, just as lung development and onset of lung function precede internal gill degeneration.

4. The sequence of events associated with the degeneration of the internal and external gill filaments is the same.

5. Cellular differentiation of the lungs is complete at the time of metamorphic crisis. All normal tissue elements are present. Primary septa formation has occurred. The development of the secondary and tertiary septa, however, occurs during post-metamorphic life.

6. Histological evidence confirms a respiratory function of the lungs in the pre-metamorphic tadpole (Helff, '31).

205. FURTHER STUDIES ON REGENERATION IN THE TAIL FINS OF *FUNDULUS HETEROCLITUS* EMBRYOS. S. Milton Nabrit, Atlanta University.

Six-day-old (Oppenheimer stages 24-25) *Fundulus* embryos were removed from their chorions by the Nicholas technique. The distal two segments were removed in cold-blooded Ringer's solution by cutting with cold steel. Some fish were kept in Ringer's for 12 hours and then transferred to sea water; others were kept in Ringer's for 24 hours and then transferred to a 4/5 Ringer's and 1/5 sea water mixture.

Tail fins differentiated in both sets, but earlier in the latter ones (7-9 days). Birnie reported that he failed to obtain differentiation of caudal fins in 65 days after cutting 5-day-old embryos.

Ages computed in days cannot be accurately compared if the temperatures are different. Healing in Ringer's may produce less sloughing of tissue than healing in sea water. If the natatory fold does not heal over the wound, rays will not differentiate readily. As the fish grows older, the fold regresses and the expectancy for fin differentiation would be less.

206. FURTHER STUDIES ON REGENERATION IN THE CENTRAL NERVOUS SYSTEM OF *FUNDULUS HETEROCLITUS* EMBRYOS. S. Milton Nabrit, Atlanta University.

The Nicholas technique for removal of *Fundulus* embryos from their chorions was employed on 3-day-old (Oppenheimer stage 20-21) fish. Large areas were removed from the forebrain with De Wecker's scissors in cold-blooded Ringer's solution. Many cells in the area of the wound escaped into the medium, and very frequently the anterior end of the nervous system was depleted of its cells. If the wound heals, the brain area may be secondarily partly refilled from areas as

far removed as the middle of the spinal cord. Some histological preparations showed that after 6 days following removal of the forebrain, the middle of the cord had been deprived of its nuclear layer entirely and only the fibrous portion of the cord was intact. In no case did this cell depletion extend into the tail bud region, which at this time was in a rapid state of proliferation.

The replacement of the central cells in the nervous system following cold steel ablation was quite similar to that observed following cautery except that cells degenerated in situ following cautery while the cells flowed into the Ringer's solution following ablation. The cells in process of migration may show a connection as a part of a network. This histological picture was presented during healing, though usually the cells appeared as elements separated by injury.

207. SURFACE TENSION OF THE ALLANTOIC AND AMNIOTIC FLUIDS OF THE DEVELOPING CHICK. Paul A. Walker, University of Connecticut.

Surface tension changes occurring in these fluids in individual chicks have been studied using the tensiometer method of DuNoüy. The allantoic fluid has a value of about 55 dynes per centimeter on the seventh day, decreasing to a minimum of 49.5 on the thirteenth day. This is related to the increase in nitrogenous materials occurring at this time. After this, a rise occurs to about 56.2 on the sixteenth day. This may perhaps be explained on the basis of pH changes, resulting in the formation of different protein complexes. From then on, surface tension again decreases to about 54.2 on the nineteenth day. This decrease may also be related to an increase in nitrogenous materials.

The tension of the amniotic fluid on the seventh day is relatively low (about 50.3 dynes per centimeter). By the ninth day, it increases to about 53.7, a level which is then maintained until the fifteenth day. This indicates a decrease (not predicted by chemical evidence) in the amount of organic materials (possibly sugars) present during early incubation. By the sixteenth day, a rise to a maximum of 56.2 occurs, and this is followed by a decrease to values of about 53.0 during the last few days of incubation. This is probably related to absorption of water from this fluid by the embryo.

All these values are lower than the surface tension of water. The significance of this fact in the distribution of solutes is discussed in relation to absorption.

208. RECIPROCAL HETEROPLASTIC CHORIO-ALLANTOIC TRANSPLANTATIONS OF MACERATED CHICK AND DUCK KIDNEY TISSUE. Carl J. Sandstrom, Herman N. Eisen and Robert S. Siffert, University College, New York University.

Macerated metanephric tissue from (a) duck embryos 21 and 24 days' incubation, and 1-, 4-, and 8-day ducklings, and (b) chick embryos of 16 and 20 days' incubation, 5-day chicks, and adults, was implanted on the chorio-allantois of 9-day chick and 13-day duck embryos, respectively.

Thirty-nine per cent of the chick hosts receiving duck tissue from embryonic stages died at various intervals after implantation whereas 96% receiving tissue from hatched stages died within 36 hours. The mortality in the former group was comparable to homotransplantations; the increase in the latter was attributed to a drastic anaphylactoid reaction caused by the sudden development, at the time of hatching, of a true species specificity factor in the protoplasm which was re-

leased by maceration. No increase in mortality occurred when chick tissue was implanted on the duck. Only 43% and 39% of the hosts which received tissue from pre-hatched and post-hatched stages, respectively, died.

No changes in the mortality rate of the hosts occurred after transplantation of intact tissue, regardless of the age of the donor tissue, or the host-donor relationship (previously reported). The reaction against the intact tissue (lymphocytes and connective tissue) was relatively mild—a response to heterotoxins, etc., of the persistent tissue, but in contrast to the transplantation of macerated tissue, the duck tissue was readily incorporated into the chick membrane with little delay, whereas chick tissue was slowly and poorly incorporated into the duck membrane.

209. RETROGRESSION OF THE AVIAN MESONEPHROS IN CHORIO-ALLANTOIC GRAFTS.

Carl J. Sandstrom, University College, New York University.

The retrogression of the chick mesonephros was studied by transplanting mesonephric tissue from donors ranging in ages from 4 to 16 days' incubation, to the chorio-allantoic membrane of chick hosts 8 and 14 days' incubation. Grafts were removed at various intervals. Twenty-four hours before removal, trypan blue was injected into the air chamber, and its presence in the cells and lumina of the secreting tubules was considered an indication that the engrafted tissue had been functionally incorporated.

Since the transplanted tissue was able to grow, differentiate, and function on the membrane, at a time when the host's mesonephroi were retrogressing and the metanephroi were rapidly increasing their functional capacities (i.e., 18 to 20 days), it was concluded that normal physiological changes in the development of the host are not factors bringing about retrogression. Poor vascularization and inability to dispose of its products induced a precocious retrogression in mesonephric tissue only 9 and 12 days old, suggesting that in normal development a diversion of the blood stream from the mesonephroi to other growth centers, or the occlusion of collecting tubules or ducts, could act as initiating mechanisms. The actual process, however, is independent of external factors, for retrogression in the grafts which were functionally incorporated, started at the appropriate time, and showed conditions at removal identical with those of equally aged controls, irregardless of the conditions extant in the host, thus corroborating Dantchakoff's ('24) observations.

210. THE EFFECT OF TESTOSTERONE PROPIONATE ON THE EARLY DEVELOPMENT OF THE REPRODUCTIVE DUCTS IN THE FEMALE SPARROW HAWK (*FALCO SP. SPARVERIUS*). Olin E. Nelson and Robert M. Stabler, University of Pennsylvania.

Injections of testosterone propionate (Schering) were made subcutaneously in the ilio-pubic area or into the pectoral muscles of young Sparrow hawks. The injections were begun 3-7 days after hatching and were continued at 3-4 day intervals until the young hawks were about ready to leave the nest, soon after which they were autopsied. Each injection was of 0.5 cc. dosage and contained approximately $12\frac{1}{2}$ mg. of the crystalline testosterone propionate. Each bird received on the average ten injections. The left oviduct responded by showing a great increase in muscularity, and an increase in the epithelial and sub-epithelial structures within the oviduct. The right oviduct showed some hypertrophy. Little change was noticeable with regard to the ovaries.

211. THE EMBRYONIC AND POST-NATAL DEVELOPMENT OF THE FEMALE PROSTATE GLAND IN THE ALBINO RAT. John J. Mahoney, State University of Iowa. (Introduced by Emil Witschi.)

The prostate of the female rat arises from a double primordium which first appears in the 19-day-8-hour embryo: a horseshoe-shaped pad of stromal tissue at the neck of the bladder, which becomes the intertubular stroma of the gland, and from one to five buds of the urethral epithelium which develop into the tubular parts. These latter grow toward the stromal pad during later embryonic stages.

During the early post-natal life the distal ends of one or two of the epithelial cords branch and form anastomoses within the stromal area. Lumina appear in the cords about the tenth day. Maximum development of the gland, characterized by tubules distended with secretion and with columnar or cuboidal epithelium, is reached about the thirtieth day. Light cytoplasmic areas appear in the epithelial cells above the nucleus which lies basally.

Secretory activity in the gland ceases after 30 days of age. The epithelium undergoes involutional changes and the relative amount of stroma increases in prostates of older females. The gland also becomes thinner, but there is no other macroscopic sign of glandular atrophy.

One or both of the embryonic prostate primordia may appear during development of females of non-prostate strains. However, the epithelial cords never enter the stromal area and no true prostate is formed.

The prostate gland of the female rat is homologous to a part of the ventral lobe of the male rat prostate. Usually it is comparable to one-fifth of the male lobe.

212. REPRODUCTIVE PHENOMENA IN THE MINK (*MUSTELA VISON*). Robert K. Enders, Swarthmore College.

The mink (*Mustela vison*) has one short breeding season per year. Isolated females fail to ovulate. While a few females fail to ovulate after copulation ovulation tends to occur 42 to 52 hours after copulation. Copulation may last for more than 2 hours but intromission as brief as 5 minutes may result in impregnation. Ovulation may follow a struggle with a male even though intromission does not take place. This indicates that ovulation is not spontaneous in the mink, and that the stimulus necessary to induce ovulation is not great. Ovulation is followed by pregnancy or pseudo-pregnancy. In our colony pregnancy lasts from 42 to 58 days.

213. THE INFLUENCE OF NERVE FIBERS UPON TASTE BUDS DURING EMBRYONIC DEVELOPMENT. Theodore W. Torrey, Indiana University.

Fibers of the developing glossopharyngeal nerve reach the basal level of the developing tongue in the rat at the beginning of the sixteenth day of gestation. By the twentieth day such fibers have formed a sub-epithelial plexus beneath the newly-forming vallate papilla, from which time they gradually penetrate the papillary epithelium. Taste buds differentiate in the walls of the papilla from the ninth day post-partum on, thus their appearance is considerably preceded by their nervous supply. Cauterized papillae in adult rats regenerate devoid of

taste buds if their functional nervous connections have previously been severed. Taste buds also fail to differentiate in intra-ocular implants of papilla primordia of all pre-taste bud ages. The results are interpreted as favoring the concept of morphogenetic dependence of taste organs upon their nervous supply.

214. EFFECTS OF PITUITARY EXTRACT UPON THE TURTLE ADRENAL CORTEX. L. T. Evans, B. Armstrong and C. M. Spencer, University of Missouri.

Thirteen *Chrysemys picta bellii* received a total of from $\frac{1}{4}$ gm. to $\frac{1}{2}$ gm., dry weight, of mammalian pituitary extract administered as daily injections for from 4 to 35 days. Six were retained as controls. The glands were fixed, stained at 5μ and stained by standard techniques.

Two methods were used to determine effects upon the cortex. The first was to count the nuclei in unit areas using an ocular grid. Over 200 such units were studied for each specimen of cortex. Second was the paper weight method in which random areas of cortex were projected on paper, drawn, cut out and weighed; numbers of nuclei were noted in each area.

Using these two methods as a check against each other it was found that the cortical cells of the adrenal of turtles which received injections were between 4% and 33% greater in size than those of controls.

The cytoplasm of many cortical cells of those injected was much vacuolated. Careful study indicated, however, that certain cells of the cortex of stimulated glands were unvacuolated and resembled closely many cells of cortex of control turtles.

Rhythms of activity possibly occur in the turtle cortex during which certain cells seem immune to hormonal stimulation. No sexual differences were observed in the cortical response to injections. Medullary tissue showed no consistent response to pituitary injections.

215. HYPOPHYSECTOMY IN THE BOX TURTLE, *TERRAPENE CAROLINA*. Ira B. Hansen and Mildred L. Tabb, George Washington University.

Removal of the pituitary has been accomplished by trephining through the roof of the mouth in both male and female turtles. Preliminary studies on the females show a drastic effect upon the ovaries. Those from hypophysectomized individuals averaged 0.61 gm. in contrast to those from unoperated controls averaging 6.0 gm. At the time of the operation, the right ovary in each case was removed. The right ovaries at operation averaged 2.7 gm. Three months later the left ovaries averaged only 0.61 gm. This shows not only an arrested development but a regression of the left ovary in spite of the fact that the right ovary is usually heavier than the left in the turtle. These operations were performed at a time when the ovarian weights in normal animals were increasing. Detailed studies of the genital tract and the relation of hypophysectomy to the other endocrine glands are in progress.

216. THE PITUITARY GLAND OF THE SOUTH AFRICAN CLAWED TOAD (*XENOPUS LAEVIS* DAUDIN). Irving Levenstein and Harry A. Charipper, Washington Square College, New York University.

The reported sensitivity of the South African clawed toad to anterior pituitary hormones has stimulated interest in the endocrine and reproductive systems of

this form. The present report concerns itself with a study of the pituitary glands from laboratory acclimated specimens of *Xenopus*. The tissues were fixed in Champy's fluid and stained with Masson's reagents (Severinghaus).

The pituitary gland, located ventral to the diencephalon, appears as a flattened disc-like structure with lateral elongate ovoid projections. In median sagittal section the disc portion resolves itself into three layers. A nervosa portion most dorsad, a pars anterior layer most ventrad and posterior with a pars intermedia as the middle stratum. It is this latter portion of the gland which extends as two wing-like lateral projections, cupping the anterior.

The pars anterior exhibits large eosinophilic cells which together with chromophobic and basophilic elements form nests bounded on all sides by delicate connective tissue partitions. An area of differentiation occurs in this part of the gland as a clearly demarcated layer of cells beginning at the posterior tip and continuing ventrally and anteriorly. This strip contains all three varieties of cells. These cells are smaller in size than those in the rest of the pars anterior. The pars intermedia is thick and contains cells slightly basophilic except in its wing-like projections where the cells are intensely blue and granular. The nervosa is made up mostly of fibers. A layer of large cells forming a columnar epithelium occurs on the surface nearest the diencephalon. Well-formed mitochondria and Golgi may be found in the different cells of the pars intermedia and anterior. Secretory granules are also present in many of the cells of the anterior.

217. THE PITUITARY BODY OF THE AFRICAN LUNG-FISH, *PROTOPTERUS AETHIOPICUS*.

Alden B. Dawson, Harvard University.

This study is based on two male specimens, 14 inches long. In *Protopterus* as in *Lepidosiren* (Kerr, '33) the anterior lobe is caudal in position but it is not sunk into a depression in the cranial floor as is that of *Lepidosiren*. In the adult *Ceratodus* (Griffiths, '38) the pars anterior secondarily takes up an anterior position and is directed vertically into a deep sella of the skull floor. The structure of all three glands closely resembles that of the amphibia.

In a ventral view of the brain only the flattened, ovoid pars anterior is visible. The infundibular recess is wide and thin-walled except in a medial, caudal region applied to the pars intermedia, where a considerable thickening occurs. The epithelial portion of the gland possesses a conspicuous hypophysial cavity which separates an anterodorsal intermediate zone from an elongated ventrocaudal pars distalis. The pars intermedia is composed of open or closed epithelial diverticula which are associated with the hypophysial cavity and are interdigitated with the compact fibrous portion of the infundibular process. The pars distalis is composed of cords of epithelial cells.

The glands were fixed in formol-sublimite and stained with Heidenhain's azan. The majority of the intermedia cells are lightly basophilic. Scattered cells react with orange G. In the pars distalis the basophiles are deeply stained and tend to be segregated from the acidophiles, being found chiefly in a medial, ventral longitudinal band extending the whole length of the anterior lobe. Laterally acidophilic cells predominate. Some react specifically with orange G, others with azocarmine.

218. FUNCTIONAL ACTIVITY OF ANTERIOR PITUITARY GRAFTS IN THE ADULT MALE GUINEA PIG. M. Schweizer, H. A. Charipper and W. Kleinberg, Washington Square College, New York University.

Hypophysectomized male guinea pigs with intra-ocular transplants (homoplastic) of anterior pituitary tissue had well-maintained reproductive systems.

The occurrence of all stages of spermatogenesis in the testes, the epididymides well filled with sperm and the presence of motile sperm in ejaculates indicated maintenance of the germinal epithelium.

Although the interstitial tissue of the testis was less abundant than in the normal guinea pig, the individual cells of this tissue were normal in appearance. There was definite evidence of androgenic activity in the large size and distended state of the seminal vesicles, the size of the horny styles and the positive results of the electrical ejaculation test.

The germinal and interstitial tissue of the testis was maintained to the same extent whether the implant tissue was obtained from a male or from a female donor.

Previous work (Schweizer, Charipper and Haterius, '37) has shown that such transplants in the hypophysectomized female guinea pig apparently secreted only follicle stimulating hormone.

It is concluded on the basis of the present investigation that intra-ocular transplants of anterior pituitary tissue in addition to secreting FSH, probably also secretes LH in amounts below the threshold necessary to affect the ovary but sufficient to stimulate the more sensitive (Greep, '37) male gonad.

219. ON THE TOXICITY OF ESTROGENS IN ADRENALECTOMIZED ANIMALS. Robert Gaunt and Abraham Edelmann, New York University.

It has been found in several laboratories that estrogens are toxic to adrenalectomized animals (rats and ferrets), whereas the androgens are non-toxic and non-beneficial, and progesterone and desoxycorticosterone are life-maintaining. It has been at least twice suggested (Cavanaugh and Gaunt; D'Amour and Funk) that the deleterious action of estrogen was possibly due to its ability to inhibit the pituitary, and thus to superimpose an hypophyseal deficiency upon an adrenal insufficiency.

This hypothesis was tested by injecting a life-reducing dose of estrin (100 I.U. Amniotin daily) into 30-day-old adrenalectomized rats of both sexes simultaneously treated with Squibb anterior pituitary extract (0.5 to 1.0 cc. daily). The extract contained growth and other metabolism-stimulating factors; it was weak in gonadotropic content and hence did not induce the complication of excessive endogenous estrogen, particularly in males. There was, however, no difference in the life-span of sixteen animals thus treated and in those receiving Amniotin only. Such results fail to confirm the above-stated hypothesis.

220. FUNCTIONAL CAPACITY OF ADRENAL CORTICAL TRANSPLANTS AT VARIOUS SITES AND ADRENAL FUNCTION IN 'WATER INTOXICATION.' W. J. Eversole, Abraham Edelmann and Robert Gaunt, New York University.

A comparison of the functional efficiency and ease of preparation of six differently situated types of adrenal cortical transplants was made in 131 adrenalecto-

mized rats. Functional capacity was determined by the ability to maintain life and by the reaction of the animals to high doses of water. Autoplastic grafts of a single adrenal capsule were made.

Grafts to the ovary, kidney and intestinal mesentery were all of approximately equal efficiency, maintaining life in nearly all cases and offering some although not normal protection against excess water. Because of the greater ease of preparation, transplants to the kidney are probably the ones of choice for routine work.

Transplants to the eye were more difficult to obtain, would maintain life when established, but afforded little, if any, protection against 'water intoxication.' Liver and muscle grafts, as used here, were rarely successful.

Cortical tissue allowed to regenerate in situ from an enucleated adrenal capsule was more efficient than any of the grafts tried. No type of transplant produced complete normality in response to excess doses of water, and evidence is offered that this is purely a cortical and not medullary deficiency.

Unlike ovarian transplants, the function of cortical tissue is not impaired by having a site (intestinal mesentery) with hepatic portal drainage.

221. THE EFFECTS OF SEASONS AND THEELIN UPON THE FEMALE GENITAL SYSTEM OF *ANOLIS CAROLINENSIS*. Llewellyn T. Evans and Mary L. Clapp, University of Missouri.

Eight adult females (2.5 gm.) and eight immature females (1.43 gm.) received an average of 10 r.u. of theelin (Parke Davis). Five adult females (2.3 gm.) and ten immature females (1.44 gm.) were retained as controls. This experiment extended from November to January, 1936. Five adult females were spayed in February, 1936, and were studied along with eight normal adult females during late May and June. These animals were killed June 12, 1936.

The oviducts of adult females injected with theelin averaged 9.3 mg. and those of immature injected females averaged 3.52 mg. Those of the adult control females averaged 6.37 mg. and those of immature control females averaged 0.41 mg.

In terms of dosage units of theelin per gram of body weight, the immature injected females showed a 16.2% greater increase in weight than the oviducts of the mature injected females. The ovaries of adults treated with theelin averaged 0.65 mg. and those of the immature injected averaged 0.43 mg., while the ovaries of adult controls averaged 0.83 mg. and those of immature controls averaged 0.29 mg.

The oviducts of summer controls averaged 53% larger by weight than those of winter controls. The oviducts of spayed females averaged 4.32 mg. while those of summer controls killed at the same time averaged 13.33 mg. The cloaca were highly folded and thick-walled in the summer controls but showed gradual diminished folding and thickness in the other series in the following order: winter theelin, winter controls, spayed.

222. RELATION OF THYROID EXTRACT TO PUGNACITY AND ANOXEMIA IN *ANOLIS CAROLINENSIS*. Llewellyn T. Evans and Mary L. Clapp, University of Missouri.

Seven males and one female each received (intraperitoneally) an average of 118 mg., dry weight, of thyroid extract divided into eight daily injections. Five

males and three females were retained as controls. Environmental factors other than thyroid injections were identical for both groups.

A total of ninety-seven encounters was studied, sixty-nine by males and twenty-eight by females. The reactions of each lizard were observed, first in the role of resident and then as non-resident.

The territorial reaction time of injected lizards as residents averaged 0.533 minute and the average number of non-responses was 1.7. The control lizards as residents showed an average reaction time of 2.74 minutes and their number of non-responses averaged 7.5.

It was found possible to evaluate each lizard in terms of 'units of pugnacity.' Injected lizards showed an average of 9.1 units as residents, and 4.7 units as non-residents; control lizards showed an average of 6.8 units as residents and 3.2 units as non-residents.

By modifying the technique used by Hellwig ('35) in his study of anoxemia in rats, it was found that injected lizards invariably succumbed before controls to the effects of anoxemia.

It was concluded that thyroid extract administered to *Anolis carolinensis* increases 'pugnacity' in terms of territoriality and also increases the need for oxygen. No sexual differences were observed.

223. SOME EFFECTS OF CRYSTALLINE SEX HORMONES ON THE REPRODUCTIVE STRUCTURES OF SEVERAL ANURANS. William O. Puckett, Princeton University.

Experiments are reported dealing with the effects of intraperitoneal injections of two crystalline sex hormones on the reproductive structures of the frog, *Rana pipiens* and the toad, *Bufo americanus*.

In the male frog, injections of small amounts of testosterone propionate over a 30-day period bring about a great hypertrophy of both the wolffian and the rudimentary müllerian ducts. No visible effect was noted in the testes. Theelin injected into male frogs causes a marked hypertrophy of the müllerian ducts but has no effect on the wolffian ducts and the testes.

Testosterone propionate injected into female frogs causes a degeneration of the egg masses, resulting in an early death of the animals. These experiments could not be carried sufficiently long to study the effects of this hormone on the sex ducts of the female frog. Theelin injected into female frogs caused no significant changes in any of the sex structures.

Testosterone propionate injected into male toads produces a marked hypertrophy of both the müllerian and wolffian ducts and a complete degeneration of the organ of Bidder. Theelin has no apparent effect on the organ of Bidder and the wolffian ducts but causes a slight hypertrophy of the rudimentary müllerian ducts. Experiments dealing with female toads are in progress.

224. EFFECT OF THYMUS GLAND, THYMUS EXTRACTS, AND THYMUS DERIVATIVES ON GROWTH AND METAMORPHOSIS OF AMPHIBIAN LARVAE. Albert S. Gordon, Savino A. D'Angelo and Harry A. Charipper, Washington Square College, New York University.

Approximately 700 *Rana pipiens* tadpoles (hind limb bud stage) were divided into seven groups. A first group received injections of the equivalent of one to two adult *Rana pipiens* thymi in saline. Animals of the second, third, fourth, and

fifth groups were injected respectively with 1–2 mg. thymocrescin (Asher), 0.05 cc. Hanson's extract, 0.25–0.5 mg. glutathione (Hoffman-La Roche), and 0.25–0.5 mg. cysteine HCl. A sixth group receiving injections of frog muscle in saline, and a last untreated group served as controls. All injections were given biweekly for 12 weeks.

In a second series, approximately 450 *Rana palustris* tadpoles were used. Group A received injections of 1–2 mg. thymocrescin; group B, 0.05 cc. Hanson's extract, and group C, 1–2 mg. crystalline albumen. Group D consisted of untreated controls. Each experimental animal received twenty injections over a period of 8 weeks.

All materials were neutralized immediately before injection and were administered intraperitoneally. All animals were fed a diet of boiled spinach, liver, and rolled oats throughout the experiment.

In both series, Hanson's extract was the only substance which produced a significant, although slight, acceleratory effect, this being exerted on both growth and metamorphosis. This effect became more evident in the later stages of development. The thymocrescin, frog thymus, glutathione, and cysteine injected animals revealed approximately the same rate of growth and development as the untreated controls. Those injected with muscle extract or albumen showed retardation of both processes. In no case did the thymus materials or derivatives employed inhibit the advancement of metamorphosis.

225. A STUDY OF GRAFTS OF MOUSE THYROID GLANDS IN NEWTS. A. Elizabeth Adams and Ruth M. Merwin, Mount Holyoke College.

Mouse thyroids were transplanted into the throats of *Triturus viridescens* (1) when the hosts were thyroidectomized and (2) after they had been thyroidectomized for 4 weeks or longer and had ceased molting. In series I, the complete inhibition of molting typically following thyroidectomy occurred, except in a few individuals where accessory newt thyroids were present. In series II, molting followed within 3.5 to 6 days (average, 4.8 days) except in three of nine cases where the graft remained only 3 hours in the host. After 4 weeks the molted newts were again black. Controls for series I received *Triturus* thyroids. Their skins remained normal except in two cases where the gland had dried somewhat before being grafted. Controls of series II molted following homoplastic thyroid transplants within 2.5 to 4 days (average, 3.5 days) and did not blacken again. From these data it is concluded that thyroid hormone is released from the grafted mouse and newt thyroids and induces molting in thyroidectomized newts, but mouse grafts do not establish themselves and maintain the normal condition of the skin, whereas newt grafts do.

Histological study of the grafts confirmed these conclusions. Mouse thyroids showed marked changes 3 hours after implantation; within 24 hours the colloid was gone from the follicular lumens and the cells were extremely altered. Invasion and phagocytosis of the graft by host cells began in 6 to 12 hours and within 6 to 9 weeks it had been replaced by white fibrous connective tissue.

226. AN ENDOCRINE BASIS FOR SEASONAL CHANGES IN ACTIVITY OF THE INTACT FROG'S HEART. T. J. B. Stier and Harriett E. Taylor, Harvard University.

The behavior of the intact heart of pithed frogs at temperatures above the 'normal' range (above 20°C.) was found to vary with the seasons of the year.

In fall frogs (*Rana pipiens*) a partial or a complete block of the beat occurred at 35°C. (average); in winter frogs between 26 and 27° and in spring frogs at 34°C.

An endocrine basis for the observed seasonal variation is indicated by experiments in which winter frogs (with upper limit between 26 and 27°C.), after injection with sufficient whole pituitary glands to induce ovulation in females, exhibited an upper limit between 31 and 38°C. (typical of fall and spring frogs).

The working hypothesis selected, views the observed change in behavior of the heart tissue as having been brought about through stimulation of the thyroid gland by the added 'thyrotropic principle' in the injected pituitary glands. Seasonal changes in activity of the heart at temperatures above the normal temperature range are viewed as originating in a seasonal change in the balance of the pituitary-thyroid interrelationship. A simple in vivo method involving the use of a general anaesthetic and an electrocardiograph for detecting the first signs of heart-block, is being developed for studying the nature of the seasonal changes produced in the metabolic activity of heart tissue by endocrine agencies and for an investigation of the factors involved in bringing about the 'winter' endocrine condition in frogs.

227. EXTRACHROMATOPHORIC ACTIVITIES OF THE SINUS GLAND OF *PALAEMONETES VULGARIS*. F. A. Brown, Jr., Northwestern University.

Ninety *Palaemonetes* were divided into three lots of thirty animals. Lot 1 was deprived of eyestalks and each animal had a *Carcinus* sinus gland implanted into the abdomen. Lot 2 was deprived of eyestalks and had an abdominal puncture but no gland implant. Lot 3 merely had an abdominal puncture. The death rates of the three lots paralleled one another for 4 or 5 days and then the eyestalkless ones died at similar rates, but at a distinctly higher rate than those with stalks. The molting rates in the three groups were distinctly different: lot 3 lowest; lot 1, 45% higher, and lot 2, 120% higher. The molting rates of lots 1 and 3 paralleled one another for the first 4 or 5 days, the probable duration of function of the implant (Brown, '39). The crustacean sinus gland has recently been shown to affect the viability and molt rate of the fresh water crustacean, *Cambarus*. These experiments confirm the general conclusions using a marine form.

228. SINUSGLANDECTOMY IN CRUSTACEANS WITHOUT BLINDING. F. A. Brown, Jr., H. E. Ederstrom and H. H. Scudamore, Northwestern University.

Hitherto all successful removals of the crustacean sinus gland have involved eyestalk removal and consequent blinding. One could not fully differentiate the effects of sinusglandectomy and blinding. By means of a very simple technic involving the use of a fine needle for penetrating the exoskeleton and a capillary glass pipette attached to an aspirator it has been possible to suck out the readily visible sinus glands of *Palaemonetes vulgaris* without apparent serious disturbance to other eyestalk structures and functions. Of sixteen operated animals with bilateral extirpation of the gland 9 days previously, all showed complete dispersion of the red pigment on a black background. When placed upon white, seven of these showed strong concentration of the red pigment, four showed a weak concentration, and five showed no concentration of the pigment. Physiological assays

of the stalk activities were then made. Six eyestalks from each of these three groups and six from normal animals were extracted in equal quantities of sea water and the chromatophorotropic activities quantitatively determined by injection into eyestalkless *Uca* and *Palaemonetes*. There was a direct relationship between activity of the eyestalk extract and the extent of white background response of the donor animals. The amount of gland removed thus determines the extent of red chromatophore response, with no background response of the red pigment when the gland is almost totally removed.

229. THE INFLUENCE OF ESTRADIOL ON THE SOCIAL ORGANIZATION OF FLOCKS OF HENS. W. C. Allee and Nicholas Collias, University of Chicago.

In an attempt to find the relation, if any, of estradiol benzoate (Schering's Progyon-B) to the peck-order of hens, thirteen high-ranking individuals among six flocks were given daily intra-muscular injections. All flockmates received neutral sesame oil. The dosage varied from 0.025 to 1.0 mg. Only two reversals resulted although the treatment continued from 39 to 68 days. BY, an alpha hen, lost to BB, the omega bird in her flock, after receiving 0.1 mg. for 43 days. In another flock RY, a medium-ranking bird, lost to YY, again the omega bird in her flock, after 36 days' treatment with 1.0 mg. daily. All these were in good health. No reversals involving only normal healthy controls occurred during the entire observation period of 10 months.

Aggressiveness, as shown by initial staged encounters with strange birds in neutral territory, was little affected by this treatment, if at all.

There was no effect even from extended injections of 0.30 mg. or less daily, on egg laying, weight, or size of comb. The comb of RY, which received 1.0 mg. daily, became eapon-like. She stopped laying but continued to squat before a cock. A poulard began squatting after only 3 days' treatment with this dosage and also developed the voice of a normal hen in contrast with untreated poulards.

The effect of estradiol treatment as given tends to be the opposite to that of testosterone which we reported last year. It is, however, not nearly so striking in its results.

230. THE COPPER AND NITROGEN CONTENT OF HOMARUS, CANCER AND LIMULUS SERA. William H. Cole and James B. Allison, Rutgers University.

Analyses for total nitrogen, non-protein nitrogen and copper have been made on the sera of *Homarus americanus*, *Cancer borealis* and *Limulus polyphemus*, and for urea nitrogen in the serum of *Limulus*. The copper in the sera and in the pure hemocyanins of *Homarus* and *Limulus* was determined by a simplified micro-procedure eliminating the electrolysis of the copper and involving the iodine-sodium thiosulfate reaction.

In each species the NPN/TN ratio was low, averaging 0.029 for *Limulus*, 0.043 for *Homarus* and 0.05 for *Cancer*. The NPN in *Limulus* contains only a trace of urea. The ratios of copper atoms to protein nitrogen atoms in the sera were 0.0018 for *Cancer*, 0.00215 for *Homarus* and 0.0023 for *Limulus*. In the pure hemocyanins prepared from the sera of *Homarus* and *Limulus* the ratios were 0.0022 and 0.0023, proving that all of the protein in those sera was hemocyanin.

231. COMPOSITION, pH AND FREEZING POINT DEPRESSIONS OF SOME INVERTEBRATE FLUIDS AND SERA. William H. Cole, Rutgers University and Mount Desert Island Biological Laboratory.

Analyses for Na, K, Ca, Mg, Cl and SO_4 were made on coelomic and ambulaeral fluids of *Asterias vulgaris*, *Strongylocentrotus drobachensis*, and *Cucumaria frondosa*, on blood of *Venus mercenaria*, on sera of *Homarus americanus*, *Cancer borealis* and *Limulus polyphemus* and on sea water in which each species lived. Freezing point depressions and pH of each were also measured.

For each of the fluids, the freezing point depression was the same as that of the corresponding sea water (within the error of measurement). The pH of each, however, was lower than that of sea water (8.1 ± 0.1); for the echinoderms and *Venus* from 7.6 to 7.8; for the crustacea from 7.3 to 7.5 and for *Limulus* from 6.80 to 7.4.

In sea water, the number of ions per 100 Na ions were as follows: 2.2 K, 2.27 Ca, 11.5 Mg, 116 Cl and 7.2 SO_4 . The K ratio was unchanged in *Venus* and *Cancer* but 15% and 27% higher in *Homarus* and *Limulus* respectively. The Ca ratio was higher by 10%, 18%, 57% and 60% in *Cancer*, *Limulus*, *Homarus* and *Venus* respectively. The Mg ratio was lower by 20%, 38%, 56% and 85% in *Venus*, *Limulus*, *Cancer* and *Homarus* respectively. The SO_4 ratio was lower by 15%, 30%, 43% and 75% in *Venus*, *Limulus*, *Cancer* and *Homarus* respectively. The Cl ratio was 4% higher in *Venus* but lower by 6%, 8% and 10% in *Limulus*, *Homarus* and *Cancer*.

232. DETERMINATION OF TOTAL GLUCOSE IN *RANA CLAMITANS* LARVAE DURING METAMORPHOSIS. Ralph G. Janes, Wayne University College of Medicine.

In view of the extensive pancreatic degeneration and general autolysis which occurs during metamorphosis, it was thought that carbohydrate metabolism might show disturbances. Consequently, an attempt was made to study the blood sugar levels during this phenomena. However, this did not prove to be feasible since it was virtually impossible to obtain adequate blood samples during all the stages of metamorphosis. As a possible approach to the problem the total glucose content of the tadpoles was determined.

Mature tadpoles which had fasted 6-8 weeks showed considerable variation in glucose levels with an average of 27 mg./100 gm. During the most extensive histolytic changes of metamorphosis, the glucose level was slightly elevated, but showed a subsequent fall at the end of metamorphosis. On the basis of the number of animals studied the rise in total glucose was not statistically significant, but it may indicate the occurrence of glucogenetic processes at this time.

233. THE AMOUNTS OF PROTYROSINASE, THE NATURAL ACTIVATOR OF PROTYROSINASE, AND RESPIRATION DURING THE COURSE OF DEVELOPMENT OF EGGS OF THE GRASSHOPPER (*MELANOPLUS DIFFERENTIALIS*) WHICH HAVE BEEN X-RAYED TO PREVENT THE FORMATION OF AN EMBRYO. J. H. Bodine, O. Malcolm Ray and L. D. Carlson, State University of Iowa.

Eggs of the grasshopper (*Melanoplus differentialis*) were x-rayed with a dosage of 1000 r on the sixth day after laying. This dosage has been found to prevent the formation of the embryo with no noticeable effect on the rest of the egg.

Determinations of the amounts of protyrosinase, and the natural activator of protyrosinase were made on these eggs lacking embryos. The respiration of the whole egg was also determined.

The amounts of the enzyme and activator at various stages of development of the x-rayed eggs are the same as those of the controls. The O_2 consumption of the whole egg failed to increase during prediapause subsequent to irradiation. It remained at the same level, however, until diapause normally occurs, at which time it dropped slightly. After breaking diapause by exposure to $5^\circ C.$ for 3 months the O_2 consumption of the x-rayed eggs increases daily until the fourth or fifth day and then remains constant.

234. THE METABOLISM OF THE FROG RETINA. Verlus F. Lindeman, Syracuse University.

The oxygen consumption of the frog's retina was studied with the Warburg method, supplemented with a modified Fenn manometric method. Using from two to twenty-five retina per manometer, the rate of oxygen consumption was determined for light and dark adapted retina in carefully controlled conditions of darkness and light. The rate was studied for alternating periods of dark and light, and readings were made at intervals of from 5 to 15 minutes. The light intensities tested ranged from 65 to 200 foot-candle power. All experiments were made at a temperature of $25.3^\circ C.$ The results are based upon sixty-seven experiments.

The Warburg method revealed no consistent difference in the rate of oxygen consumption in the dark as compared to light. Data was obtained with the Fenn manometer, which measured smaller changes in rate, and could be read at shorter intervals, indicates a slight increase in oxygen consumption for approximately 10 minutes after the retina is exposed to the dark, and then returns to a value which is constant for light.

In a series of ninety-three experiments at a light intensity of approximately 65 foot-candle power and a temperature of $25.3^\circ C.$, the retina was found to consume 3.223 cu.mm. of oxygen per milligram dry weight per hour.

235. THE EFFECT OF 2,4-DINITROPHENOL ON THE OXYGEN CONSUMPTION OF FROG RETINA. C. Riley Houck, Syracuse University. (Introduced by Verlus F. Lindeman.)

The effect of 2,4-dinitrophenol on the oxygen consumption of light-adapted retina of the frog, *Rana pipiens*, was determined by the direct manometric method of Warburg. Control retina were placed in Ringer's solution of M/9 NaCl, KCl, $CaCl_2$, 100:1.5:1 cc., respectively, and experimental retina in Ringer's with 2,4-dinitrophenol at various concentrations. All solutions were buffered with Sorensen phosphate buffers, M/9 Na_2HPO_4 and KH_2PO_4 to a pH of 7.5. Retina were illuminated with 65 footcandles at $25.3^\circ C.$

The Q_{O_2} of 165 control retina in Ringer's was 3.11 (Q_{O_2} equals cubic millimeters oxygen consumed per milligram dry weight per hour). Four hundred and five retina were studied at the following molar concentrations of 2,4-dinitrophenol in Ringer's solution: 2.72×10^{-5} M, 1.36×10^{-5} M, 5.43×10^{-6} M, 2.72×10^{-6} M,

and 1.09×10^{-6} M. The average percentage increase in the rate of oxygen consumption was 55.94, 49.51, 51.12, 51.44, and 44.37 respectively.

Due to the low solubility of 2,4-dinitrophenol in water, forty-five retina were studied in Na, 2,4-dinitrophenylate-Ringer's solution to obtain a higher concentration. The rate of oxygen consumption was depressed 52.44% and 64.30% at 3.07×10^{-4} M and 4.12×10^{-4} M concentrations of the salt respectively.

236. SEASONAL DIFFERENCES IN THE OXYGEN EXCHANGE AND GLYCOLYSIS OF TISSUES IN THE CLAM *VENUS MERCENARIA*. Hoyt S. Hopkins, New York University, College of Dentistry.

Muscle tissue slices in sea water, with oxygen in the respirometers, displayed an age difference in oxygen respiration rate of similar magnitude in summer and winter. The respiratory rate of gill in summer was 21.0% greater in young clams than in old, with respirometers shaken 30 oscillations per minute; 16.8% greater, even with respirometers not shaken. During winter and early spring the oxygen exchange in young clams' gill was 7.4% higher than in old, with shaking rate of 30; 6.0% lower without shaking. At a shaking rate of 60 the oxygen exchange was 13.3% higher—a figure still below that obtained in summer without shaking. Mantle tissue from young clams, in winter, showed respiration rates more than 20% greater than from old clams, thus resembling muscle.

Results of determinations on the rate of glycolysis, expressed in microliters CO_2 liberated from sea water + bicarbonate, by tissue in nitrogen, were as follows: In winter, muscle tissue of old clams 164 μl . CO_2 , young clams 161; in summer, 174 and 213 respectively. In winter, gill of old clams 117, young clams 68; in summer, 160 and 193 respectively. Mantle tissue, like muscle, showed small seasonal differences. Figures for mantle were 348 and 302, for old and young clams, in winter; 337 and 373 in summer.

These results indicate a selective condition of hibernation as regards metabolism in the gills, particularly of young clams, correlated with their sluggish activity in winter and spring.

237. THE EFFECT OF CO_2 AND LACTIC ACID ON THE O_2 -COMBINING POWER OF WHOLE AND HEMOLYZED MARINE FISH BLOOD. Laurence Irving and R. W. Root, Swarthmore College and the College of the City of New York.

In the blood of fish CO_2 greatly reduces the ability of the hemoglobin to combine with oxygen. Data are presented for the whole and hemolyzed blood of the tautog showing the change in O_2 -combining power as a function of pH, when the pH is modified by the addition of CO_2 or lactic acid. Oxygen combining power diminished in proportion as the pH was lowered by either lactic acid or CO_2 in the same way. Measurement or calculation of pH showed a reduction of oxygen combining power proportional to the decrease in pH, whether CO_2 or lactic acid caused acidification.

Whole blood was half saturated with oxygen at pH 7.0. Hemolyzed blood was half saturated at pH 6.1. The inference is that pH within the corpuscle at half saturation was 6.1. However, calculation of the pH in the erythrocyte from data on partition of CO_2 between plasma and erythrocytes indicated that pH in the

erythrocyte was not lower than 6.8. In order to refer the effect of CO_2 to acidification it is necessary to assume either that the hemolyzed blood is abnormal or that the constants used for calculation within the erythrocyte are in error by 0.8 pH. At present there is no basis for either assumption. It is also possible that CO_2 affects the hemoglobin within the erythrocyte primarily by altering other components of the solution within the cell rather than by influence upon the H ions.

238. THE TRANSPORT OF CO_2 BY THE WHOLE AND HEMOLYZED BLOOD OF THE MARINE FISH, *TAUTOGA ONITIS*, AND ITS PARTITION BETWEEN RED CELL AND PLASMA. R. W. Root and Laurence Irving, College of the City of New York and Swarthmore College.

CO_2 dissociation curves of whole and hemolyzed blood of the tautog have been established, as well as curves showing the effect of degree of oxygenation of blood on CO_2 -combining power. Above 50 mm. CO_2 tension in whole blood there is no appreciable difference in the ability of 'reduced' and 'oxygenated' blood to combine with CO_2 . Below 50 mm. CO_2 , however, 'reduced' blood definitely combines with more CO_2 than 'oxygenated,' there being a maximum difference of approximately 5 vol. % around 10–15 mm. CO_2 . On hemolysis 20–30% of the CO_2 -combining power of whole blood is lost, but hemolyzed blood shows a difference along the whole range of CO_2 tensions from 5–100 mm. in the ability of 'reduced' and 'oxygenated' blood to combine with CO_2 . The maximum difference in favor of the 'reduced' blood is again about 5 vol. %. Reasons are given for the contrast in the 'Haldane effect' in whole and hemolyzed blood, these being made evident by a consideration of the difference in the effect of CO_2 on O_2 -saturation, and by a study of the relation between CO_2 -combining power and degree of oxygenation. Preliminary data on the partition of CO_2 between red cell and plasma have also been obtained and the results considered insofar as they bear on the general problem of the role of the red cell in respiratory transport and the effect of CO_2 on the O_2 -combining power of hemoglobin in whole blood.

239. TEMPERATURE ACTIVATION OF THE UREA-SOY BEAN UREASE SYSTEM. Irwin W. Sizer, Massachusetts Institute of Technology.

Urea hydrolysis by soy bean urease was studied by measuring CO_2 evolution as a function of time, using the Barcroft differential manometer. At all temperatures between 0 and 50°C. CO_2 evolution is a linear function of time, and rate of hydrolysis was calculated from the slope of the straight line drawn through the plotted points. An Arrhenius plot of the data gave a linear relationship with a corresponding energy of activation of either 11,700 and 8700 cal. per gram mol. In certain cases a break occurred in the curve with corresponding activation energies of 11,700 below and 8700 cal. above the critical temperature. The activation energy of a particular digest may have either of the above values depending upon the source of the urease, method of purification, and the nature of the stabilizer added to the digest. The same activation energies have been obtained using unmodified urease which has not been extracted from the bean.

The activation energies of the soy bean urease-urea system are identical with those of the crystalline Jack bean urease-urea system, suggesting that the activation energy of an enzyme from different species is the same, at least for closely related legumes.

240. THE ACTION OF ACETYLCHOLINE AND ESERINE ON REVERSAL IN THE INTACT HEART OF THE ASCIDIAN, *PEROPHORA VIRIDIS*. A. J. Waterman, Williams College.

The variable number of abvisceral beats (toward branchial basket and stolon) is greater than the advisceral, and reversal is attributed to alternating dominance of terminal heart centers. Nervous tissue in the ascidian heart or connecting it with the intersiphonal ganglion has been questioned. Acetylcholine and choline esterase have not been demonstrated.

Acetylcholine (1-2500 and 1-5000) causes a temporary increase in the number of abvisceral beats, but atropin prevents this effect. The excitatory period lasts about 4-10 minutes. Some hearts fail to respond. Subsequently the number of beats in either direction becomes highly irregular, rate is affected, rest periods become irregular in length, and beating may stop without reversal. In stronger concentrations dominance ceases and uncoordinated beating occurs at both ends. 1-1000 usually stops the heart quickly and the animal contracts. After recovery in sea water rhythm may be temporarily irregular and more rapid. Atropin quickly stops activity.

Eserine slightly increases the length of the abvisceral phase, reduces the variability in both phases and stimulates a mechanically blocked heart to activity. After preliminary exposure to eserine, lower concentrations of acetylcholine are effective, while some hearts show increased response. As with acetylcholin, initial increase may be replaced by cessation of activity which then returns after washing. Thus eserine has an excitatory action and increases sensitivity to acetylcholine. These reactions resemble those of the hearts of certain invertebrates, and suggest the presence of a local nervous system in the *Periphora* heart which may be cholinergic.

241. THE BIOCHEMICAL BASIS OF THE COLORS OF THE PLUMOSE ANEMONE *METRIDIUM SENILE*. Denis L. Fox, Scripps Institution of Oceanography, University of California, and C. F. A. Pantin, University of Cambridge. (Introduced by W. R. Coe.)

Reflecting individual biochemical mutations, color-variants within this species exhibit wide differences in pigmentation from dead white through yellow, orange, pink, to deep orange-red, due to varying types and combinations of carotenoids in increasingly high concentrations; alternatively the presence of melanin compounds may direct the preponderating color through a series of pale brown, buff, gray, to deep brown or, in parts, even black.

Considerable variations exist in the colors of specific organs or tissues in intermediate variants of both the 'red' and 'brown' series, and mutual exclusiveness between carotenoids and melanins is often pronounced in given tissues.

A brief summary of the carotenoids follows:

COLOR	RELATIVE QUANTITY OF CAROTENOID	KIND OF CAROTENOID
White	Very little	Astacene esters
Red	Much	Astacene-like esters (not identical)
Ruddy-brown	Much	Astacene-like esters
Yellows, oranges	Considerable	Astacene or astacene-like esters, carotenes, xanthophylls, xanthophyll esters
Browns (various shades)	Very little	Ditto, but in varying alternatives and varying relative quantities

Pure reds and whites, while phenotypically 'albinos' possess both the chromogen and oxidase precursors necessary for the formation of melanin; their triturated tissues yield melanin in a few hours.

Purine compounds, present in all, contribute to the whiteness of the pigment-free variants. Uric acid was the only purine demonstrated in significant quantities. It was present in endodermal tissues and in yellow sheets of mucous excreta. No hematin compounds or significant quantities of flavines were detected.

242. THE EFFECTS OF PARTIAL AND TOTAL BLINDING ON THE COLOR CHANGES OF THE SUMMER FLOUNDER (*PARALICHTHYS DENTATUS*). Clinton M. Osborn, Ohio State University.

When previously white-adapted summer flounders are totally blinded the integument undergoes a very gradual darkening first changing to a pale greenish-yellow, then buff, and finally to a homogeneous intermediate brown. This sequence of events obtains regardless of the shade or color of the illuminated environment in which the fish is placed after blinding.

At the end of the experiment (5 weeks after blinding) the fishes were not maximally dark but rather, intermediate. This is unlike the catfish and many other teleosts.

However, if flounders are black-adapted previous to total blinding the operation does not affect their shade. They remain permanently (5 weeks at least) dark brownish-black, the shade typical of black-adapted specimens. The shade of the illuminated environment does not influence this reaction. Such a fish, if artificially paled by the injection of adrenalin, subsequently darkens completely to its original shade rather than stopping in the intermediate condition.

If but one eye (dorsal or ventral) is removed the adjustments to backgrounds are unaffected qualitatively but in most cases the rate of adaptive change is greatly retarded especially during the first 10 postoperative days. Some specimens which were black-adapted and then semi-blinded required four times as long to white-adapt as in tests previous to the operation. Although semi-blinding previously white-adapted flounders had less effect on black-adaptation than on the reverse change, the rate was obviously retarded. Considerable variation was encountered.

243. ACTIVITY OF MELANOPHORES IN TWO LIZARDS, *PHRYNOSOMA DOUGLASSII* AND *P. MODESTUM*. Madelene E. Pierce, Vassar College.

Normal time for blanching (*P. douglassii*, 1–1.5 hours; *P. modestum*, 2–3 hours) and for darkening (*P. douglassii*, 1 hour; *P. modestum*, 1–2 hours) was determined. In *P. douglassii* the criterion used was the condition of the dentate scales and of the dorsal scales upon the head and legs. In *P. modestum* the entire dorsal surface of the body was the criterion. Since the color change occurs very slowly after the more rapid activity of the first $\frac{1}{2}$ hour, the determination of exact end points was difficult.

The reactions of the melanophores of each species were studied under the following conditions: (1) absence of light, (2) high and low temperatures with varying backgrounds, (3) electrical stimulation, (4) nerve cutting, (5) excitement from teasing, (6) enucleation, (7) injections of adrenalin extract from other animals, (8) injections of blood from other animals, (9) anesthetics.

Behavior of these two species of *Phrynosoma* does not agree completely with that of other species reported formerly. Of these two species, *P. modestum* is much more reactive to changing conditions.

244. ACTIVITY OF MELANOPHORES IN A TELEOST, *MOLLIENESIA LATIPINNA*. Madelene E. Pierce, Vassar College.

The melanophores in the skin of *Mollienesia latipinna* consist of two groups. One group serves as general ground color with the melanophores more numerous in the dorsal than in the lateral region. The other group forms seven longitudinal stripes on each side of the body. When in response to a background the fish darkens, the stripes stand out more clearly even though the ground melanophores also disperse their pigment. When in response to a white background the fish blanches, the ground melanophores contract, the stripes fade, and the fish becomes pale olive. Reactions to background are rapid, darkening requiring 10–25 seconds and blanching 25–50 seconds.

The reaction of melanophores to temperature, absence of light, enucleation, and anesthetics was studied.

The normal temperature for *Mollienesia* is about 25°C. Below 10°C. the fish become extremely sluggish; and above 35°C. they die. Between 10–12°C. the fish became anesthetized, and a slight dispersion of pigment, regardless of background, occurred. At 35°C. the melanophores responded normally to white and black backgrounds.

In the absence of light, or after enucleation, the melanophores assumed an intermediate phase regardless of temperature.

Under the influence of ether or chloroform, the melanophores disperse pigment. If first injected with adrenalin, the melanophores remain contracted.

245. A PRELIMINARY STUDY OF THE LIGHT REACTION OF THE ROCK BARNACLE, *BALANUS BALANOIDES*. D. E. Minnich, University of Minnesota.

The rock barnacle, *Balanus balanoides*, is extremely sensitive to decreases of illumination as noted by Cole ('32). To an appropriate decrease of illumination active animals, viz. animals carrying on regular rhythmic movements of the cirri,

respond by an immediate retraction of the cirri and closure of the valves. To an increase of illumination there is no such response. The length of the period of closure following a decrease of illumination depends upon the magnitude of the decrease. To a decrease from 24.5 to 23 f.c., seven animals given five trials each averaged 2.74 seconds of closure. To a decrease from 53 to 23 f.c. the same animals in the same number of trials averaged 9.91 seconds of closure. As expected, preliminary experiments also showed a relation between the length of light adaptation and the magnitude of the decrease of illumination necessary to effect a response. Thus in animals continuously exposed to 23 f.c. an exposure for as brief an interval as 0.05 seconds to 53 f.c. sufficed to effect a response in some animals. Under similar conditions, however, an exposure of ca. 8 seconds to 24.5 f.c. was required to produce a closure in the most sensitive animals.

246. TWO TYPES OF RHYTHMIC ELECTRICAL RESPONSE TO ILLUMINATION OF THE EYE IN THE GRASSHOPPER, *MELANOPLUS FEMUR-RUBRUM*. K. D. Roeder, Tufts College.

Silver electrodes made contact with drops of electrode jelly on the compound eye and top of the head. Filters were used to cut out axon spikes and very slow potential changes. If the eye was illuminated a regular train of waves at a frequency of 25-35 per second were superimposed on the -c wave of the retinogram. They ceased abruptly if the light was turned off, and disappeared after 8-15 seconds if illumination was continued. If the previous period of darkness was less than a few seconds or longer than 30 minutes, the rhythm was reduced or absent. The rhythm is not affected by median separation of the protocerebral ganglia, and simultaneous records from both compound eyes shows that two separate rhythms occur. Section of the optic tract abolishes the rhythmic response, though the various components of the retinogram are still present. This rhythm was invariably obtained from normal insects, and is thought to be due to synchronized firing of neurones in the ipsilateral protocerebral ganglion.

A different rhythm was obtained occasionally in unoperated insects, but only after long periods of dark adaptation. It appeared more frequently after section of the optic tract or injury to the optic ganglion, and consisted of a train of larger, faster (50-80 per second) waves which followed illumination of the eye, but lasted for less than 1 second. This faster rhythm apparently had its origin in the optic ganglion, and is thought to occur only under abnormal conditions, whereas the protocerebral rhythm appears to be a normal phenomenon.

247. SOME FURTHER EXPERIMENTS ON THE RELATION OF THE EXTERNAL ENVIRONMENT TO THE SPERMATOGENETIC CYCLE OF *FUNDULUS HETEROCITUS*. J. Wendell Burger, Trinity College.

The normal rate of testicular involution of *Fundulus* which follows the breeding season was duplicated in laboratory fish by keeping the temperature of the water at levels approximating those that occurred in nature (17°-29°C.). Testicular involution was considerably retarded by somewhat lower temperatures (11°-19°C.). This together with other studies indicate that the velocity of the annual sexual cycle is in part at least controlled by temperature.

248. THE EFFECT OF DIFFERENT RELATIVE HUMIDITIES ON THE DEVELOPMENT OF THE EGG, AND THE WEIGHT AND WATER CONTENT OF THE LARVAE OF *CALLOSAMIA PROMETHEA*. Daniel Ludwig, University College, New York University.

Eggs of the promethea moth were collected daily and subjected to different relative humidities, ranging from 0 to 96%, and at temperatures of 25°, 27.5°, and 30°C. The duration of the egg stage, per cent hatching, and the weight and water content of the larvae were determined at each combination of temperature and humidity.

At 25°C., at all humidities, most of the eggs completed development although the percentage of hatching varied from 95.2 at 76% humidity, to 3.2 at 0%. At lower humidities, the larvae ate through the chorions but many were unable to hatch. It appears that the chorion protects the larva from desiccation at low humidities, since after it has been punctured, desiccation occurs too rapidly to permit emergence. The average weight of the larvae decreased with humidity from 1.48 mg. at 96% to 0.84 mg. at 0%. Water content also decreased, averaging 78.0% at 96% humidity and 58.3% at 0% humidity. Embryonic development required from 9 to 10 days at 25°C., regardless of humidity.

At 27.5°C., no larvae were obtained at humidities of less than 27%. At this temperature development required 8.8 days at 76%, and 10.0 days at 27% humidity. At 30°C., no larvae hatched at humidities of less than 76%, development requiring 8.3 days.

249. OSMOTIC REGULATION IN SEVERAL PACIFIC COAST CRABS. Lowell L. Jones, University of California. (Introduced by S. F. Light.)

Regulation in an aquatic animal may be said to be hyperosmotic or hypoosmotic, according to whether the blood has a higher or a lower osmotic pressure than the water in which the animal is immersed.

A study was made of osmotic regulation in nine species of crabs, only one of which had been previously investigated. Three, *Cancer antennarius*, *Lophopanopeus heathii*, and *Speocarcinus californiensis*, were found to be without regulation, the blood being isotonic with the water. The other six species were all found to have hyperosmotic regulation in brackish water, the degree of regulation (magnitude of blood-water difference) being in the order named (in an ascending scale): *Cancer magister*, *Rhithropanopeus harrisi*, *Hemigrapsus oregonensis*, *Hemigrapsus nudus*, *Pachygrapsus crassipes*, and *Uca crenulata*. In full-strength or more concentrated sea water the first four species in the list showed no regulation, whereas the last two were hypoosmotic. *Uca*, with the greatest degree of hyperosmotic regulation, ranks highest also in hypoosmotic regulation.

A close correlation exists between these rankings and tendency toward life in the air, semi-terrestrial species being characterized by hypoosmotic regulation in normal or higher concentrations of sea water and a high degree of hyperosmotic regulation in brackish water.

250. PATHS OF WATER EXCHANGE IN THE EARTHWORM. A. V. Wolf, Department of Physiology, School of Medicine and Dentistry, University of Rochester. (Introduced by E. F. Adolph.)

The rate of water excretion by the nephridia of *Lumbricus terrestris*, estimated from weight increases of worms undisturbed while immersed in distilled water,

was $2.55 \pm 0.14\%$ of the basal body weight per hour ($\pm$ indicates standard error). This was determined by first handling worms (rolling with filter paper) to empty their reservoirs and bring them to basal weight. Loss of weight on handling was ascertained to be mainly due to discharge from the nephridia.

The rate of gut excretion, as determined by collection of the fluid in cannulas inserted into mouth and anus, was only one-tenth of the total excretion. Diurnal fluctuations of gross and basal weight (3.2 ± 0.3 and $2.3 \pm 0.3\%$ of basal weight, respectively) might represent variations in gut and nephridial contents.

Repeated handlings of worms whether ligated at both ends or unligated, indicated that nephridia could discharge from their reservoirs about $13.4 \pm 0.9\%$ of the basal weight of fluid. From this the storage capacity of a single nephridium was estimated at 0.048% of basal weight. At a single handling the nephridia discharged an average of $63.1 \pm 2.4\%$ of the dischargeable fluid in the nephridia at that time.

The initial rate of intake of water through the integument by dehydrated worms increased with the amount of deficit of water. Worms lost approximately 18% of their body weight of water before the amplitude of spontaneous bodily contractions diminished.

251. THE TOXIC EFFECTS OF WHEAT ON THE METAMORPHOSIS OF THE JAPANESE BEETLE. Daniel Ludwig, University College, New York University.

Groups of Japanese beetle larvae were reared in Carex mold to which grains of wheat were added for different periods of time during larval development. Of the larvae surviving 70 days of the third instar the percentage which pupated was recorded for each group. Wheat is beneficial during early larval development, but is toxic to individuals approaching metamorphosis. Its maximal beneficial effects were obtained when it was fed throughout the first, second, and a part of the third instar but removed before the larva was ready to begin metamorphosis. If wheat was fed until the end of the first 30 days of the third instar, 32.7% of the larvae surviving 70 days of this instar pupated. However, if wheat was fed until 70 days of the third instar, only 2.7% survived pupation. This toxic effect of wheat appears to be counteracted by the presence of soil in the culture medium. When larvae were fed Carex mold mixed with soil and with the addition of wheat grains throughout the life cycle, more than 50% of those surviving 70 days of the third instar molted to the pupal stage. When Carex mold and wheat were present throughout the larval period, but when all old wheat was removed and fresh wheat was added every 5 days, 44.9% of the larvae surviving 70 days of the third instar pupated. Hence, it appears that toxic substances result from the decomposition of wheat. Soil might produce its beneficial effects by the adsorption of these substances.

252. PRELIMINARY NUTRITIONAL STUDIES ON THE REPRODUCTION OF TRIBOLIUM CONFUSUM IN A SYNTHETIC MEDIUM. Thomas Park and William Burrows, The University of Chicago.

In preparing the ground work for future nutritional studies with the flour beetle, *Tribolium confusum*, a synthetic medium has been developed. The basis of this medium is a fine wood dust which, after extraction with hot alcohol, drying

and sifting serves as an adequate physical milieu for the beetles but which is apparently inert nutritionally. Nutrient added in the form of casein, dextrose, Osborn-Mendel salt mixture and dried yeast permits development of the beetle in all stages. It has been shown that (1) *Tribolium* fecundity and fertility in the synthetic medium is not greatly different from that of beetles grown in certain cereal flours, and (2) stock populations can be reared successfully in the new medium. The usefulness of this technique in analyzing the relation of certain food elements to reproduction, post-embryonic development and mortality is suggested.

253. ENERGY REQUIREMENTS FOR REPRODUCTION IN BIRDS. S. Charles Kendeigh, University of Illinois.

Experimental studies with caged English sparrows, *Passer domesticus*, show that increasing the length of day by adding artificial light stimulates gonads to develop, but more so in males than in females. Added exercise in place of light produces some regression of gonad size in males but has no effect in females. Restriction of feeding time or exposure to low temperature did not modify the effect of added light. The initial development of gonads seems not to be conditioned by energy resources. However, in the female the complete development of the ovaries to egg-laying is energy demanding. Analysis of over 2000 sets of house wren, *Troglodytes aedon*, eggs indicate that the size of the egg, number laid per set, time of laying, and whether any eggs are laid at all are affected by the modifying influence of temperature on the energy resources of the adult bird especially during the 3 days preceding laying.

254. THE EFFECTS OF HYDROSTATIC PRESSURE UPON GELATION PHENOMENON IN ANIMATE AND INANIMATE SYSTEMS. Douglas A. Marsland and Dugald E. S. Brown, Washington Square College and Medical College, New York University.

Previous work has demonstrated that increased pressure in the range up to 550 atmospheres, induces an extensive and reversible solation of the gelated protoplasm of a number of living cells (*Amoebae*, marine eggs and plant cells). These results led to an investigation of two inanimate gels, namely of gelatine, and of myosin derived from rabbit muscle.

The relative rigidity or 'viscosity' of the compressed gels was evaluated by a modification of the Bridgeman method. The pressure bomb is equipped with four windows which permit a determination of the velocity of a steel ball, 5/32 inch diameter, as it falls under gravity, through a glass tube filled with the gel in question.

In relation to pressure, myosin gels behave very much like protoplasmic gels, but this is not the case for gelatine. When the maximum pressure is applied to a myosin gel which has been allowed to set thoroughly, the resistance of the gel to the passage of the ball falls to about 10% of the atmospheric value. In a gelatine gel (2% gelatine in M/20 phosphate buffer, pH 6.4) the same degree of pressure augments the 'viscosity,' in certain cases up to 800%.

The pressure-induced liquefaction of the myosin gel and of the protoplasmic gels occurs rapidly, reaching a peak in less than 20 seconds. Reversal of the

change after decompression is much more rapid in the protoplasmic system, however, since 2-4 hours are required for the re-setting of a thoroughly liquefied myosin gel.

From these results it seems probable that pressure acts upon certain protein components of a protoplasmic gel directly, rather than through the medium of an excitable system.

255. THE DIFFUSIVITY OF WATER INTO HALF EGGS OF THE SEA URCHIN, *ARBACIA PUNCTULATA*.* Herbert Shapiro, Vassar College.

As was discovered by R. S. Lillie, the egg of *Arbacia punctulata* increases its permeability to water several fold when fertilized. The metabolism of 'half' eggs obtained by strong centrifuging in sea water-sucrose mixtures has also been previously investigated (Shapiro, J. C. C. P., 6, 101, '35) and it was found that the light 'halves' exhibit a Q_{O_2} similar to that of the whole eggs, and likewise show a comparable increase in oxidative activity when fertilized. The object of the present investigation was to determine the behavior of these egg fragments with respect to the relative ease of penetration of water into the cell before and after fertilization. There is an increase in total surface area when the egg is broken into two fragments. The light halves raise good fertilization membranes, and their cleavage and development is very similar to that of the whole eggs. As control experiments, a confirmation was made of the increase in permeability of the whole egg to water when fertilized, by allowing individual eggs to swell in 60% sea water at room temperatures and measuring the diameters at frequent intervals over a period of 12 to 15 minutes. The light halves again behave like the whole eggs, for on fertilization water enters the fertilized light half more rapidly, by several fold. The heavy half has also been studied in this way, but the data are as yet incompletely analyzed.

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256. COALESCENCE WITH OIL DROPS OF *ARBACIA* EGGS IMMERSSED IN KCl OR CaCl₂ SOLUTIONS. M. J. Kopac, Washington Square College, New York University.

Unfertilized eggs (jelly removed) immersed in 0.34 M CaCl₂ solutions show a higher coalescency with oil drops than similar eggs immersed in 0.53 M KCl solutions after allowance is made for the differences in tension between the oils and respective aqueous phases. Calcium apparently increases the adhesiveness of the cellular surface of unfertilized eggs. An oil drop, if brought in contact with an egg immersed in Ca solution and suddenly pulled away, will carry with it a small portion of the egg.

Fertilized eggs with their fertilization membranes removed show an entirely different effect. Thus in 0.53 M KCl, these eggs coalesce very readily with oil drops, whereas similar eggs in 0.34 M CaCl₂ coalesce less readily or not at all. The reasons for this effect are obvious since the hyaline layer which appears after fertilization is unstable in KCl, but it is completely stabilized in CaCl₂ or sea water. In the latter, the hyaline layer becomes a firm extraneous coat. In KCl, the hyaline layer is dispersed and no extraneous coat is formed.

The entry of an oil drop into fertilized eggs immersed in KCl immediately induces a wave of peripheral disintegration, leaving a mass of disintegrating cytoplasm unbounded by any surface. In the absence of convection currents, the disintegrating egg mass becomes surrounded by a halo of granules in active Brownian movement. Mechanical agitations completely disperse the contents of cytolyzed cells. Pigment and other granules are stable (at least 72 hours) when released in the KCl solution.

257. INTERFACIAL REACTIONS BETWEEN OILS AND DISINTEGRATED ARBACIA EGGS.
M. J. Kopac, Washington Square College, N. Y. University.

Unfertilized Arbacia eggs can be readily made to disintegrate in 0.53 M KCl if all extraneous coats including the vitelline membrane have been removed. A packed volume of 0.4 cc. of eggs was disintegrated in 4.5 cc. of KCl solution. Pigment and other granules were removed from the mixture by strong centrifugal forces. The resulting supernatant liquid had a brownish color and showed a strong Tyndall effect. This liquid also possessed weak elastic properties, resembling those of dilute myosin solutions.

The tensions of cottonseed oil and of oleic acid when brought in contact with the above liquid were measured by a micro-adaption of the maximum bubble pressure method (unpublished). At $\text{pH} = 7$, the interfacial tension of cottonseed oil was 5 to 6 dynes/cm., while oleic acid tensions ranged from 0.5 to 1.2 dynes/cm. Similar values were previously obtained by using the maximum bubble pressure method on intact cells.

The adsorption factor of cottonseed oil in contact with the supernate was in most cases less than 0.1 while that of oleic acid was more than 0.9. These values which indicate the amount of adsorbed protein were obtained by employing the drop-retraction method (Kopac. Biol. Bull., 75:372, '38). An adsorption factor of 1.0 would result in a spontaneous crinkling effect thereby implying zero interfacial tension. Such reactions are obtained by injecting certain oils into *Asterias* eggs at the instant of cytolysis.

On the basis of low interfacial tensions (cf. oleic acid) the surface active components from disintegrated Arbacia eggs are, in all probability, proteins. These data further indicate (1) an inverse relation between interfacial tension and protein adsorption at oil-water interfaces, and (2) variability of adsorption tendencies of proteins with different oil surfaces.

258. THE DEVAUX EFFECT IN CENTRIFUGED ASTERIAS EGGS. M. J. Kopac, Washington Square College, N. Y. University.

Asterias eggs were centrifuged for 10 to 12 minutes at 6 kilogravities in an isopycnotic solution of sugar and sea water. Such centrifugation of mature eggs yields an oil cap, a small hyaline zone, and a granular centrifugal zone. No hyaline zone appears in the cytoplasm of centrifuged immature eggs, the centripetal pole being occupied by the persisting germinal vesicle.

Micro-drops of *Oleum Percomorphum* were injected into the hyaline and the granular zones of mature eggs. Following cytolysis, the Devaux crinkling effect (Kopac. Biol. Bull., 75:351, 1938) appeared quickly (10 to 20 seconds) on all

drops injected into the centrifugal zones. Drops injected into the hyaline zones in most cases gave no Devaux effect. When the effect occurred, the crinkling rates were very low.

Although the germinal vesicle is essentially hyaline, Devaux effects were obtained there in both centrifuged and uncentrifuged eggs. The extent and rate of crinkling were frequently greater on drops placed in the germinal vesicles than on those injected into granular centrifugal zones.

The Devaux crinkling effect in the cytoplasm appears to be linked with the breakdown of the denser granules, particularly of those which accumulate methylene violet from dilute solutions in sea water. These experiments show that the hyaline substance of the germinal vesicle may be differentiated from the hyaline zone of the cytoplasm by its ability to promote crinkling on the surfaces of oil drops.

259. COMPARATIVE EFFECTS OF X-RADIATION ON ISOLATED AND NON-ISOLATED NUCLEI. William R. Duryee, Washington Square College, New York University.

Germinal vesicles from ovarian eggs of three species of frogs were irradiated both in intact eggs and after being isolated and washed free from cytoplasm in Ca-free Ringer. Irradiation dosages were graded from 500 to 50,000 r, at the calculated rate of 7,800 r/min.

Chromosomes in nuclei isolated subsequent to previous irradiation in situ showed progressive injuries starting with 1,000 r, in contrast to those in nuclei isolated first and then irradiated. The latter even after 50,000 r showed no marked differences from non-irradiated controls. No appreciable latent period was observed in any of the twenty-eight experiments.

Chromosome injuries were of three distinct types: progressive loss of side branches or chromomere loops, fragmentation of the longitudinal chromonemata and separation of the members of synaptic pairs. Contraction of the chromosomes occurred when they were exposed to cytoplasm injured by any method as previously described under the term, Autofixation.

Further support to the major conclusion that radiation damage to the chromosomes results primarily from chemical products of the injured cytoplasm is given by the fact that nuclei, having first been isolated and then placed in a concentrated egg brei and exposed to 50,000 r, showed typical chromosome defects of nuclei irradiated in situ.

260. THE EFFECT OF HYPOTONIC AND HYPERTONIC SOLUTIONS ON THE RESPIRATION OF CHICKEN ERYTHROCYTES. F. R. Hunter, Rhode Island State College.

Chicken erythrocytes placed in hypertonic solutions of NaCl respire at a lower rate than in an isotonic environment. There is roughly a linear relationship between concentration of NaCl and oxygen consumption. This suppression of respiration is not reversible. When they are placed in slightly hypotonic solutions, chicken erythrocytes respire at approximately the same rate as the control cells even when some of the cells are hemolyzed. Highly hypotonic solutions causing considerable hemolysis decrease the oxygen consumption markedly. The interpretation of these data is not as yet clear.

261. SOLUBILITY PROPERTIES OF THE SALIVARY GLAND NUCLEUS IN *DROSOPHILA*.

Ethel Glancy, Washington Square College, New York University. (Introduced by Robert Chambers.)

Salivary glands of *Drosophila melanogaster* and *D. virilis* were immersed in large volumes of alkalis, acids and organic solvents. Dilute inorganic and organic acidic solutions failed to dissolve the nuclear components. However, in alkaline solutions of monovalent cations the chromosomes first swelled, then became indistinct, and finally disappeared. The nucleolus, although visible for a short time after the disappearance of the chromosomes, also became invisible resulting in an optically homogeneous nucleus. If the immersion time had been short, the normal nuclear structure could be restored on returning the gland to a Ringer's solution. The effect was irreversible, however, after prolonged immersion. These irreversible nuclei became diffusely colored when subjected to the Feulgen test.

Lipoid solvents (xylene, benzene and brombenzene) if saturated with water dissolved the chromosomes but not the nucleolus. Bile salts at pH 7 and above also dissolved the chromosomes, but produced no visible effect on the nucleolus.

The lipo-protein nature of chromosomes is indicated by the dispersive action of nucleoprotein and lipid solvents. The nucleolus, on the other hand, is relatively non-lipoidal as shown by its insolubility in fat solvents.

262. STUDIES ON HUMAN SPERMATOOA. I. RESPIRATION OF HUMAN SPERMATOOA.

L. B. Shettles, Department of Obstetrics, Johns Hopkins University and Hospital.

The rate of respiration of human spermatozoa varies inversely with the age of the specimen and directly with the number of cells per unit volume. There is no oxygen consumption by semen devoid of spermatozoa. The respiratory quotient of human spermatozoa varies inversely with the age of the specimen and directly with the rate of oxygen consumption, which indicates a shift in the metabolites being oxidized. The age of the spermatozoa and the number of cells per unit volume may not be the only factors involved in the consumption of oxygen, for the results obtained indicate that individual variation is also a factor.

263. STUDIES ON HUMAN SPERMATOOA. II. RESPONSE OF HUMAN SPERMATOOA TO VARIOUS GASES. L. B. Shettles, Department of Obstetrics, Johns Hopkins University and Hospital.

Human spermatozoa remain very active for several hours in pure nitrogen, nitrous oxide, helium, and air reduced to very low pressure; in helium and likewise in oxygen, there is marked increase in activity. On the other hand, carbon dioxide produces complete immobility within a few minutes, the exact time varying with the source and the age of the specimen, with the frequency of ejaculation, and with the cells within a given specimen. The toxic effect of the gas is dependent neither upon its acid character, nor upon anoxia, but upon other properties. Motility of spermatozoa is restored when the carbon dioxide is replaced, immediately after all movement ceases, by nitrogen, air, or oxygen, but fewer and fewer cells are restored after each additional exposure. Ether and alcohol vapors produce quiescence, but if the spermatozoa are exposed for more than a few minutes, they cannot be revived.

264. STUDIES ON HUMAN SPERMATOOA. III. FACTORS INFLUENCING THE RESISTANCE OF HUMAN SPERMATOOA TO VERY LOW TEMPERATURES. L. B. Shettles, Department of Obstetrics, Johns Hopkins University and Hospital.

There is marked individual variation in the sensitivity of human spermatozoa to -79° , -196° , and -269.5° C., respectively, but the magnitude of the change in temperature, over the range tested, does not seem to be an important factor in resuscitation. The longer the time after ejaculation, the more sensitive the spermatozoa are to freezing. The effect of the freezing temperature varies directly with the length of exposure. Spermatozoa which do not survive freezing are not fragmented, but those which are revived from very low temperatures remain active for several hours.

265. THE RELATION BETWEEN STRYCHNIN SULPHATE, ATTACHMENT TO THE SUBSTRATUM AND FREQUENCY OF INGESTION OF FOOD BY AMOEBA PROTEUS. R. A. Fennell, Michigan State College.

In solutions of 0.0029 M NaCl, 0.0029 M KCl, 0.002 M CaCl_2 or 0.002 M MgCl_2 containing 0.000069 M strychnin sulphate percentage attachment of *Amoeba proteus* to the substratum was 83.3 in MgCl_2 , 68.0 in CaCl_2 , 2.5 in KCl, and 1.3 in NaCl. But the frequency of ingestion of chilomonads by the amoebae was highest in NaCl (46 in 20 minutes), lower in KCl (45 in 20 minutes), lower in CaCl_2 (38 in 20 minutes), and lowest in MgCl_2 (31 in 20 minutes).

Frequency of ingestion is therefore not closely correlated with attachment to the substratum.

In the NaCl, KCl, or CaCl_2 solutions many chilomonads adhere to the plasmalemma of the amoebae, but in MgCl_2 solution only a few adhere to it. The strychnin appears to make the surface of the chilomonads sticky in the former solutions but not in the latter. Frequency of ingestion is correlated with this.

Frequency of ingestion by dark adapted amoebae in MgCl_2 solution containing strychnin varies directly with luminous intensity. Percentage of disintegration of amoebae from agitation in solutions containing strychnin is 88 in NaCl, 81 in KCl, 78 in CaCl_2 , and 60 in MgCl_2 .

266. THE HISTORY OF THE ACROBLAST IN ANASA TRISTIS. Arthur W. Pollister and Norman Tardiff, Columbia University.

Hirschler described late spermatids of *Palomena* (Pentatomidae), with small cup-like Golgi bodies in the elongating tail sheath while there was still an acroblast (larger Golgi body) in the sperm head. He believed these tail sheath Golgi bodies to be newly formed structures. Taking exception to this explanation, Gatenby later reported that in another Pentatomid, *Anasa tristis*, similar extra Golgi bodies were not formed "de novo" but were merely "unsuccessful" elements that had been excluded from any role in acrosome formation, when but a single one of the Golgi bodies of the early spermatid enlarged to become the definitive acroblast. This was a contradiction of Bowen's classical description in Pentatomidae of the origin of the acroblast by fusion of all the Golgi bodies. We have re-examined *Anasa tristis*, using excellently impregnated slides made by the highly specific Mann-Kopsch technique; and we find complete confirmation

of the findings of both Bowen and Hirschler. Gatenby overlooked a stage before spermatid elongation, when there is unquestionably but a single large acroblast and not a trace of other Golgi bodies. The structures which Gatenby, from his examination of Flemming-hematoxylin preparations, called unsuccessful Golgi bodies do not impregnate with osmic acid, and hence they are certainly not Golgi bodies. The stage figured by Hirschler is part of the process of breaking up of the acroblast, for when the small Golgi bodies appear in the tail sheath the acroblast has already begun to decrease in size. The process of acroblast disintegration in *Anasa* is like that described by Johnson in the orthopteran, *Oecanthus*.

267. DICTYOKINESIS IN VERTEBRATE SOMATIC TISSUES. Arthur W. Pollister, Columbia University.

The behavior of the Golgi apparatus at cell-division (dictyokinesis) is reported in the hepatic epithelium and leucocytes of larval *Ambystoma*, in the superficial gastric epithelium of the ranid tadpole, and in the neck cells of the Crypts of Lieberkuhn of the mouse. The following cycle is noted in all these cases. In late prophase the single complex lamellar structure breaks up into small granules which cluster around the future spindle poles. During metaphase and early anaphase the amount of Golgi substance is greatly decreased (sometimes to complete disappearance). In late anaphase there is a sudden increase in the osmophilic Golgi granules which then are evenly scattered throughout the cytoplasm of the daughter cells. Earlier observers who have likewise noted this deficiency of Golgi substance at metaphase and early anaphase have interpreted it as due to technical difficulties in its demonstration at these stages. An alternative explanation is that the old Golgi apparatus entirely disappears and that the granules of late anaphase are entirely newly formed. Overlapping of these two processes would obscure the earlier one, and thus account for the fact that stages with complete absence of Golgi substance are rare. During telophase the osmiophilic granules progressively fuse, finally resulting in a single complex Golgi platework in the position characteristic of the resting cell. It is especially noteworthy that at an intermediate stage in this fusion the Golgi substance is temporarily in the form of a number of cup-like bodies, in size and shape resembling the most common condition in invertebrate tissues; thus affording strong evidence that the Golgi substance is essentially similar in all animal cells.

268. GROWTH OF FOLLICLES IN THE OVARIES OF THE BAT, *MYOTIS GRISESCENS*, Mary J. Guthrie, University of Missouri, and Katharine R. Jeffers, Duke University.

The follicular population of 90 females collected throughout the year was counted. Using cytological criteria, follicles were identified as growing or retrogressing and classified into size groups on the basis of maximum diameter, correlated with degree of differentiation. The average number of growing follicles per ovary is lowest at the time of parturition in June and highest at the end of August. There is a rapid rise in total follicles per ovary on the average during early lactation and a sharp decline as lactation continues. Larger follicles,

however, increase in number during this period; their retrogression is correlated with involution of the mammary gland. Follicles in which an antrum appears are most numerous early in September; they do not occur at other seasons. By late September, as the period of hibernation approaches, only one remains, the follicle of ovulation. When the average volumes of the oocytes and of their associated follicle cells in the different size groups are calculated and the ratio follicular volume/oocyte volume plotted against the average diameter of follicles in the groups, it is apparent that oocytes and follicles grow at different rates as the egg-follicle system differentiates. This growth relationship exhibits considerable seasonal variation. All of these observations are interpreted in terms of a single hypophyseal hormone to which oocyte and follicle cells have a varying sensitivity, with resultant competition for available supply within the ovarian population and between it and receptive cells in other organs, such as the mammary glands.

269. ERYTHROPHAGOCYTOSIS IN CHICKS REARED ON AN IRON-TREATED VITAMIN E DEFICIENT RATION SUPPLEMENTED WITH HALIBUT LIVER OIL. F. B. Adamstone, University of Illinois.

Chicks reared from hatching on an iron-treated vitamin-E deficient diet supplemented with halibut liver oil react differently from chicks fed the same ration supplemented with cod or sardine liver oil. The characteristic lesions found in the latter group do not occur but instead, at autopsy after 3 months, no visceral lesions were found except swollen friable livers marked with numerous brown nodules. Also, the marrow of the long bones was very compact. Histological studies showed: 1. In the brown nodules the liver cells were enlarged and swollen and the sinusoids greatly widened. Tests with potassium ferrocyanide or ammonium thiocyanate demonstrated the presence of ferric iron in these cells either as distinct granules or diffused through the cytoplasm; 2. An extraordinary mobilization of monocytes and Kupffer cells was evident in the liver sinusoids. These phagocytes contained erythrocytes in various stages of disintegration. Hence the iron deposits are probably derived from the destroyed erythrocytes rather than from the iron used to treat the food; 3. The compactness of the marrow was due to marked increase in myeloid tissue and decrease in adipose tissue in the marrow thus compensating for destruction of erythrocytes in the liver.

Although some decided difference between halibut and cod or sardine liver oils is indicated, no satisfactory explanation for the reaction can be given. It is suggested, however, that some injury to the erythrocytes may be involved which is related in some way to the destruction of anti-oxidants associated with vitamin-E.

270. THE EFFECTS OF VITAMIN E DEFICIENCY ON THE TADPOLE OF RANA PIPPIENS. Edna Mae Pratt, University of Illinois. (Introduced by F. B. Adamstone.)

Tadpoles were fed an iron-treated vitamin E deficient ration from the time of hatching. Metamorphosis was delayed and usually completely inhibited. Tadpoles which failed to metamorphose grew until extremely large. Metamorphosis

was induced by thyroid feeding and by intraperitoneal injections of thyroid, anterior pituitary substances, and in one case, wheat germ oil.

Histologically the thyroid gland presented a completely inactive or slightly active picture.

The anterior lobe of the pituitary of the E-free in comparison with the controls showed no apparent differences in basophilic or oxyphilic cells. However, the E-free gland showed cells with little cytoplasm, thus producing a compact mass of nuclei suggesting secretory inactivity.

The oldest E-free male tadpoles had mature testes, and E-free females had ovaries containing extremely large ova.

The skin of E-free tadpoles was very pale due to a reduction of the "free" melanin in the epithelial cells and a contraction and reduction in the number of epidermal melanophores with a loss of pigment granules. The deep melanophores tended to be completely or partially contracted. The xantholeucophores were expanded. Pituitrin injections caused a restoration toward the normal condition.

It is concluded that the anterior hypophysis is partially inactivated, thus failing to stimulate the thyroid gland to release its hormone but allowing growth to continue. Likewise the pigmentary condition is probably due to inactivation of the intermediate lobe.

Lack of degeneration of the gonads suggests the ability of this organ to resist the effects of E-deficiency during the tadpole stage.

271. FIBRILLOGENESIS FOLLOWING THE EXPERIMENTAL PRODUCTION OF TUBERCULOSIS IN THE TESTIS OF THE GUINEA PIG. E. Joseph Karolyi, University of Minnesota. (Introduced by A. R. Ringoen.)

Using the procedure outlined by Baitsell and Mason, tuberculosis of the testis was produced. There followed a degeneration of the germinal epithelium of the seminiferous tubules with a concurrent fluid as well as cellular exudation into the intertubular areas; the formation of typical caseous tubercles and a copious production of collagenous tissue.

Fibrillogenesis could best be followed at the periphery of the tubercles. Mallory's connective tissue stain was used to follow the extent of fiber formation. The newly formed fibrous tissue was closely associated with elongate fibroblast-like cells. When stained with Mallory's phospho-tungstic acid haematoxylin, thin blue-black fibers were found within and at the surface of the fibroblast-like cells. These fibers mingled inextricably with the easily recognized collagenous fibers. The intra-cellular nature of these blue-black fibers can best be demonstrated at 14 days post inoculation. In certain regions these fibers leave the cells and extend for long distances. Connections between the intra-cellular fibers and the extra-cellular collagenous fibers were found.

The conclusions drawn conform with those of Mall ('02), Lewis ('17), and Jordan ('39) who also found that the precursors of collagenous fibers arise intracellularly. The opinion of Baitsell and Mason, who contend that in the testis of the guinea pig following experimental tuberculosis connective tissue fibers arise as a transformation of fibrin fibers in a cell free exudate, is contradicted by the evidence derived from this study.

272. GROWTH OF THE CILIATE, COLPIDIUM CAMPYLUM, IN "INCOMPLETE" PROTEIN MEDIA. R. P. Hall, New York University.

In earlier attempts to grow Colpidium in gelatin medium, successful results were obtained with a mixture of gelatin, dextrose, pimelic acid and KH_2PO_4 . More recently, strains have been isolated in a medium containing gelatin and certain inorganic salts. The oldest strain has passed 16 transfers and is over 9 months old. Hence, no added stimulant or vitamin is essential to growth of Colpidium in gelatin. However, population density is seldom one-fifth as large as in stock culture media, and attempts to determine possible organic and inorganic deficiencies are in progress.

Several strains have been isolated in silk-peptone media; the oldest has been maintained for 22 transfers over a period of 13 months. Hence, silk-peptone, without added growth factor, is adequate for Colpidium, although population density is always less than one-third of that in stock media. Silk-peptone "de-thiaminized" (pH 9.6, at 122°C .) 20 minutes supported very slow growth for eight transfers; addition of thiamine raised population density nearly to that in untreated peptone. In peptone de-thiaminized 90 minutes the effect of thiamine was 50-75% as great, although Colpidium lived only one transfer without thiamine. Addition of a mixture of 10 amino acids to de-thiaminized (20 minutes) peptone raised the population density, although much less than thiamine. The results suggest that heating peptone in alkaline solution to inactivate thiamine may also change other constituents, and that these effects may be counterbalanced to a limited extent by subsequent addition of amino acids.

273. THE NITROGEN AND CARBON REQUIREMENTS OF LOBOMONAS PIRIFORMIS. Kenneth L. Osterud, New York University. (Introduced by R. P. Hall.)

An earlier report, that Lobomonas piriformis is autotrophic, has been confirmed. As nitrogen sources, NH_4NO_3 , KNO_3 , and a combination of NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$ have supported growth for a number of serial transfers. Hence, either ammonium salts or nitrates are satisfactory.

Early indications that glycocoll or asparagine could serve as the nitrogen source are also confirmed, since strains started from a culture in inorganic medium have passed the tenth transfer in these organic media.

Growth in several proteins and protein derivatives has been determined. Difco preparations, arranged in order of efficacy, are as follows: 'tryptone,' isoelectric casein, gelatin and 'protone.' Whereas 'protone' is of doubtful value, the others are greatly superior to inorganic or amino nitrogen.

Although several amines appear to be poor substitutes even for nitrate or ammonium salt, certain ones (methylamine, allylamine, ethylamine, glucosamine) have supported slow growth for at least four transfers (a period of 12 weeks or more). Acetamide is more effective, supporting ten successive transfers in this period. Urea and guanidine are toxic in concentrations used.

Growth in darkness requires a suitable carbon source (acetate, or soluble starch). An ammonium nitrate and acetate medium has supported growth for three transfers (12 weeks) in darkness. Glycocoll plus acetate is satisfactory, while tryptone plus acetate (or starch) is a superior medium for growth in darkness.

Lobomonas piriformis thus possesses a labile nutritive mechanism, and is capable of a variety of different types of nutrition, including photoautotrophic and heteroautotrophic. The possibility of nitrogen-fixation is now under investigation.

274. GROWTH OF *EUGLENA GRACILIS* ON INORGANIC NITROGEN SOURCES IN THE ABSENCE OF LIGHT. Henry W. Schoenborn, New York University. (Introduced by R. P. Hall.)

Euglena gracilis is known to be photoautotrophic; that is, in light it can grow in purely inorganic media. However, previous reports indicate that this species requires organic nitrogen sources for growth in darkness. At present, an attempt is being made to culture *E. gracilis*, in darkness, in three different media, each containing an inorganic nitrogen source and an organic carbon source (e.g., sodium acetate). The serial transfer technique is being employed, and the first transfer in each experimental medium was inoculated with an autotrophic strain which had been grown in light for about 7 months on inorganic nitrogen salts. Thus, even in the first transfer in darkness, there was no significant concentration of organic nitrogen. In two media, growth has been observed through four successive transfers covering a period of 18 weeks. The increase in number of flagellates was slight during the fifth transfer, and in the sixth and seventh no significant growth was observed. It appears probable that the media being employed will not permit indefinite growth of *E. gracilis* in the absence of light; however, since growth was observed through four transfers (18 weeks), it is difficult to conclude that this species is incapable of utilizing inorganic nitrogen sources in darkness. The possibility remains that the solutions used may be deficient in certain elements, and that with more adequate media *E. gracilis* may be capable of continuous growth in darkness.

275. DIVISION OF *PARAMECIUM TRICHIUM* IN A COMPARATIVELY CONSTANT CULTURE OF HAY INFUSION. Ralph Wichterman, Temple University.

Mass cultures. Mass cultures were begun from isolations of *Paramecium trichium* collected in Philadelphia as follows: $\frac{1}{2}$ gm. of hay was boiled in 250 cc. of distilled water for 10 minutes, allowed to ripen while covered for 24 hours then innoculated. Large numbers were present 6-7 days after innoculation which was followed by a fairly rapid decrease in numbers. After about 12-14 days, relatively few active forms were found in a culture; instead great numbers of 'pseudo-cysts' were seen. These 'pseudo-cysts' bear a striking resemblance to ciliate cysts in general appearance. They are somewhat smaller than active paramecia, roughly spherical with a thick irregular wall, conspicuous macronucleus, and granular protoplasm with no differentiated cytoplasmic structures. Attempts at excystation proved unsuccessful. Many could be found in various stages of degeneration.

Isolation cultures. Active individuals were isolated from a rich culture and placed in small culture dishes containing about six drops of ripened hay infusion and kept in moist chambers at about 22°C. Generally four lines were going at one time and examinations and transfers were made daily. One line passed through 125 generations during 98 days, making an average daily fission-rate of 1.3. The average fission-rate for all the lines started until their extinction was 1.65 divisions per day. The fastest daily fission-rate was 3.75 while the slowest was one division in 7 days. The longest non-fission interval followed by death was 19 days.

At the beginning of a decreasing fission-rate, there is an increase in size of the ciliate but as the rate of division decreases further, the animals become smaller and thinner. A slowing down of the fission-rate results in a slowing down of the movement of the paramecia while a faster fission-rate resulted in greater activity.

276. STABLE AND UNSTABLE CYSTS IN *WOODRUFFIA METABOLICA*. Willis H. Johnson, Stanford University.

Two types of permanent cysts are formed by this carnivorous ciliate. The primary cause of encystment in each case is the absence of food. The stable type resembles the permanent cysts of Colpoda and several other ciliates in that an organic substrate such as hay infusion is required for excystment. The unstable cyst differs from the stable type in that excystment can be induced with distilled water or fresh salt solution, or, in the case of those formed at 30°C., by lowering the temperature to 20°C. without any change of medium. Stable cysts are viable after long periods of drying, whereas drying is fatal to the unstable type. At least one liquid vacuole is present in each unstable cyst while none is found in the stable type. Unstable cysts were viable after ageing 4 months.

Unstable cysts are not formed in normal cultures at 20° to 25°C. By increasing the temperature unstable cysts are induced and at 35°C. all cysts are unstable. Also, increasing the salt content of the medium from 0.012 to 1.2% results in the formation of 100% unstable cysts. At pH 5.6 about 75% of the cysts formed are unstable. Other factors which influence the formation of unstable cysts are low temperature (10°C.), low oxygen tension and crowding.

It appears that the unstable type of cyst may be caused by any one of several unfavorable conditions, coupled with the primary cause of encystment.

277. THE RELATIVE VOLUME OF STORAGE GRANULES IN THE CILIATE *ICHTHYOPH-
THIRIUS*. R. F. MacLennan, State College of Washington.

The study of changes in cytoplasmic granules is hindered by the lack of convenient methods for determining the relative volume of granules and hyaloplasm. Centrifugal stratification is convenient if the cell has a regular shape as in eggs. This method was extended to cells of irregular shape by the use of capillary centrifuge tubes of such a bore that when the ciliate is forced into the tube by centrifugation it is molded into a cylindrical shape without rupturing the cell membrane and at the same time the granules are stratified. The volume of each layer may then be calculated from the inner diameter of the tube and the length of each layer. The standard centrifuge head is replaced with a rigid bar to the ends of which are rigidly attached cups lined with balsa wood. The capillary centrifuge tubes when inserted in suitable holes in the balsa wood are cushioned from vibration but are held rigidly so that stratification is at right angles to the long axis. The tubes are drawn out to have an open end of 1-2 mm. tapering down to a short segment of even bore, 50-200 μ in diameter. The ciliates are placed in the open funnel and when centrifuged are gently forced through the tapered section into the cylindrical part. A series of mature *Ichthyophthirius* gave the following results: neutral fat and fatty acid 8.9-9.5%; protein and glycogen 25.3-26.8%; macronucleus 2.6-5.5%; hyaloplasm 58.7-62.3%.

278. OBSERVATIONS ON *NOSEMA NOTABILIS* N. SP., PARASITIC IN A MYXOSPORIDIAN.
R. R. Kudo, University of Illinois.

The trophozoites of a myxosporidian inhabiting the urinary bladder of a toad fish, *Opsanus tau*, of the Chesapeake Bay, are frequently infected by a microsporidian. The host myxosporidian appears to be *Sphaerospora polymorpha* Davis (1917), although certain morphological characteristics of the spores differ from those described by Davis. The microsporidian is distributed throughout the cytoplasm of the host protozoan. As seen in life the spores are ovoid, with unequally rounded extremities and enveloped by a single-piece spore membrane. The spores measure $3-4\ \mu$ long by $2-2.5\ \mu$ in diameter. Often many spores fill the cytosome of a trophozoite of the host myxosporidian completely in which case the nuclei of the latter protozoan appear to have undergone degeneration. In such a trophozoite of the myxosporidian there is no complete spore-formation. The schizogony is by binary or multiple fission and each schizont transforms itself into a sporont which in turn develops into a spore. Neither the host myxosporidian nor the microsporidian has been found in the tissues of the urinary bladder of the toad fish. It appears certain that this myxosporidian which is considered a new species, develops solely in the myxosporidian body. No schizonts or spores have so far been found to occur in the sporoplasm of the myxosporidian spore, it is therefore considered that new infection begins by accidental contact of the two endosporidians in the urinary bladder of the toad fish.

279. ATLANTIC COAST POLYCLADS. Libbie H. Hyman, American Museum of Natural History.

Study of all available material, including that of Pearse, very erroneously determined, reveals twenty-four species and one subspecies of polyclads on the Atlantic coast of the United States and Canada. Acotylea: Discocelidae (*Coronadena mutabilis*, n.g.), Plehniidae (*Discocelides ellipsoides*), Latocestidae (*Latocestus whartoni*), Stylochidae (*Stylochus ellipticus*, *oculiferus*, *zebra*, *inimicus*, and *pulcher*, n.sp.), Leptoplanidae (*Stylochoplana angusta*, *Notoplana atomata*, *Euplana gracilis*, *Eu. carolinensis*, n.sp., *Digynopora americana*, n.g., n.sp.), Hoploplanidae (*Hoploplana inquilina*, *H. i. thaisana*), Planoceridae (*Gnesioceros floridana*, *Planctoplanella atlantica*, n.g., n.sp.), Enantiidae (*Enantia pellucida*). Cotylea: Pseudoceridae (*Thysanozoon nigrum*, *Thy. Brochi* (?), *Pseudoceros maculosus*), Euryleptidae (*Eurylepta maculosa*, *Oligoclado floridanus*, *Acerotisa baiae*, n.sp.), Prosthlostomidae (*Prosthlostomum lobatum*). This list excludes the polyclads peculiar to the floating Sargassum. The most interesting of these species are *Coronadena mutabilis*, having a semicircle of large prostatic organs around the male genital atrium; *Planctoplanella atlantica*, a new pelagic polyclad from the vicinity of Beaufort, N. C.; *Digynopora americana*, a new leptoplanid having two female pores; and *Enantia pellucida*, with marginal spines, belonging to a genus and family of which hitherto only one species was known, not seen again since its discovery in 1890.

280. STUDIES IN NOCTURNAL ECOLOGY. IX. FURTHER ANALYSIS OF ACTIVITY OF PANAMA RAIN FOREST ANIMALS. Orlando Park, Albert Barden and Eliot Williams, Northwestern University.

The quantitative and experimental results of a preliminary expedition have been reported (Park, '38). In the present study vocalization under natural conditions of twenty species resident in the neotropical rain forest of the Panama Canal Zone were quantitatively charted against time, light intensity, air temperature and relative humidity. Nine were nocturnal, nine diurnal and one arrhythmic. The species included a typical cross section of insects, frogs, birds and mammals. There was a striking positive correlation between normal species-vocalization, local weather and the 24-hour cycle of environmental periodic influences, with a maximum of vocalization at dawn and at dusk. A collateral part of the work was a series of activity records obtained by objective, mechanical, recording equipment on a toad, *Bufo marinus* and a marsupial, *Marmosa isthmica*. The data from these two series are discussed with reference to the rain forest community.

281. AN APPLICATION OF THE BERGMANN ZOÖGEOGRAPHIC PRINCIPLE. Orlando Park, Northwestern University.

The nocturnal, carnivorous, forest inhabiting carabid beetle, *Dicaelus purpuratus*, ranges through the deciduous forest formation from northern New York and Michigan to extreme southern Louisiana and peninsular Florida. A series of specimens over this area was measured to obtain specimen square area units. These units were plotted against latitude of collecting locality and the result was a curve of positive correlation between increasing size and increasing proximity to southern limit of the range of the species. These and other ecological data on the species are discussed.

282. SURVIVAL SEQUENCE OF FISHES LIVING IN IMPERMANENT POOLS. Myron Gordon, New York Aquarium.

A study is being made of the competition between the species of fishes which occupy the impermanent ponds that lie within the Rio Papaloapan basin of Oaxaca, Mexico. During the rainy season, roughly May to October, the overflow of the larger rivers, Tonto and Papaloapan, fills inland depressions to form a series of disconnected pools later on. When the rains subside, November to April, pools are formed within the small stream beds. By intensive seining in these restricted bodies of water at intervals during the last stages of their existence, some idea may be gained of the factors that determine survival. The number of individuals and their average size is being estimated for the total population. In one pond, collections were made on March 3, 6, 7, 10, 1939. The population consisted of predatory fishes: large *Belonesox* and *Cichlosoma*. Non-predators were: *Astyanax*, *Hemigrammus*, *Platypoecilus*, *Xiphophorus*, *Gambusia*, *Mollienisia*, *Rivulus* and *Pseudoxiphophorus*; some large *Rhamdia* were present. The average size of all members decreased as the dry season advanced. Toward the end, the predatory and non-predatory fishes were represented chiefly by their young. The outstanding agents in eliminating the larger fishes were kingfishes, egrets and

herons. Some species of fishes, *Platyphacellus*, reach their greatest population density in small ponds. Often all are lost by elimination of their habitat. The completely dried-up ponds are resupplied with water and their quota of fish species from permanent reservoirs, the larger rivers.

283. A METHOD FOR THE DETERMINATION OF ARSENIC IN WATERS. C. E. Brambel and R. P. Cowles, Johns Hopkins University.

The micro-gutzeit procedure has been adapted for the estimation of arsenic compounds in estuarine waters. Readily reproducible results are obtainable provided the conditions under which the test is performed are not varied.

Values obtained with this method are in close agreement within experimental error with those given by photometric methods. A series of known concentrations of arsenic including a blank are prepared at the same time with the unknown sample. Each test is set up in triplicate. Curves obtained from such series of known concentrations of arsenic are readily repeated: the curve is linear and the slope deviates negligibly, when successive experiments are performed at different times.

Cahill departed from the usual gutzeit procedure with the use of sensitized cotton threads in place of the regularly adopted paper strips. As a consequence the sensitivity of the method was greatly increased and at the same time maintaining a high degree of accuracy. The increased sensitivity has made this method available for the estimation of the minute concentrations of arsenic found in estuarine waters.

The following precautions are to be strictly adhered to (1) control of temperature during all reactions: precooling bath 5°C. and final bath 10°C., (2) ten mesh granulated zinc metal for generation of hydrogen, (3) dipping of sensitized rayon threads in saturated potassium iodide (to accentuate stain and facilitate its measurement).

Concentrations as low as 5 mg. arsenate or arsenite (or a mixture of both) arsenic per cubic meter of water may be determined with an accuracy of plus or minus 1 mg.

284. PHOSPHORUS AND ARSENIC CONTENT OF THE WATERS OF UPPER CHESAPEAKE BAY. R. P. Cowles and C. E. Brambel, Johns Hopkins University.

Tests for inorganic phosphate phosphorus and arsenate arsenic have been made for the surface inshore waters of the upper part of Chesapeake Bay during the late summer of 1939. The inorganic phosphorus and the arsenate arsenic has been determined by the photometric method.

The average phosphate phosphorus in five regions in the upper part of the bay averaged 5 mg. per cubic meter, but in the region of the Baltimore Disposal Plant the value increased from 50 to 150 mg. per cubic meter. The arsenate arsenic in the same five regions ranged from 0 mg. to 3 mg. per cubic meter. But in the region of the Baltimore Disposal Plant the arsenic content was considerably higher (14 mg. per cubic meter.)

285. TWO SPECIES OF TREMATODES FROM A DEEP SEA SCORPION FISH, *SEBASTES MADURENSIS*. R. F. Nigrelli, New York Aquarium.

Sebastes madurensis inhabits the region around Madeira Islands and may be taken in 50 fathoms of water. Four stomach and two intestinal parasites were found. The stomach trematodes are hemiurids, belonging to the genus *Tubulovesicula* Yamaguti, and differ from previously described species in total length; body-tail ratio; size of testes and ovary; size and shape of the seminal vesicle; extent of pars prostatica; and size of eggs. The name *Tubulovesicula madurensis* is designated for this species.

The intestinal parasites are allocreadids, belonging to the genus *Podocotyle* (Duj.). They differ from other known species in total length, pharynx-esophagus ratio; distribution of vitellaria; shape and size of ovary; shape, size and position of the seminal vesicle. The name *Podocotyle atzi* is designated for this species.

286. LIFE HISTORY STUDY OF A MONOGENETIC TREMATODE. Allard Anthony Paul, College of the City of New York and New York Aquarium. (Introduced by R. F. Nigrelli.)

The life history of a member of the subfamily Diplectaninae Monticelli infesting the gills of the River Puffer (*Tetraodon fluviatilis*) at the New York Aquarium is the subject of this experimental study. The presence of the parasite was detected by filamented tetrahedral eggs discovered at the bottom of tanks in which the hosts were isolated. The eggs hatched at the end of 5 days and the larvae, which are ciliated, were studied in both the living and stained conditions. Infestation of three specimens of *T. fluviatilis* has been accomplished and a number of stages in the development of the parasite are being secured for further study.

287. *ENDOSPHAERA ENGLEMANNI*, ENDOPARASITIC IN *TRICHODINA* INFECTING THE PUFFER, *SPHEROIDES MACULATUS* (LINNAEUS). Morton Padnos, New York University and New York Aquarium. (Introduced by R. F. Nigrelli.)

Between the months of August and the early part of October, 1938, an epidemic of *Trichodiniasis* occurred among puffers (*Sphaeroides maculatus*) around New York and New Jersey waters.

Upon examination a small and large form were found in the gills. Approximately 80% of the smaller form and 1% of the larger form were secondarily infected with *Endosphaera englemanni*. This sucktorian parasite has previously been reported in fresh water species of *Vorticella*, in *Didinium nasutum* and *Trichodina pediculus*. The infection of the *Trichodina* in the present material apparently establishes for the first time the presence of the parasite in marine *Trichodina*. The morphology and life history of this endoparasite is similar to that reported by Lynch and Noble ('31) for *E. englemanni* parasitic in a species of *Vorticella*.

In its parasitic existence, *E. englemanni* penetrates its host, undergoes a growth stage and reproduces by 'budding.' During this process it distorts the shape of the host's macronucleus and causes the chromatin granules of the macronucleus to coalesce and form large 'chromatin islands' in the macronuclear matrix. Concomitantly the ground substance of the macronucleus becomes light staining. *Endosphaera* apparently affects the normal cellular activities of its host and is parasitic during any stage of the life history of *Trichodina*.

288. THE MORPHOLOGY AND LIFE HISTORY OF TRICHODINA INFECTING THE PUFFER, *SPHAEROIDES MACULATUS* (LINNAEUS). Morton Padnos, New York University and New York Aquarium. (Introduced by C. M. Breder.)

From August to the early part of October, 1938, eighty out of ninety-one, or 90% of the puffers (*Sphaeroides maculatus*) taken from New York and New Jersey waters were infected with *Trichodina* in the gills. The majority of the infected gills were parasitized with a small turban-shaped ciliate. Some samples were doubly infected, and contained in addition a larger hemispherical form. It appears from a statistical determination of various measurements that these are two distinct forms.

A survey of the various morphological characters of *Trichodina* infecting other genera of fish, reported by previous investigators, indicates a significant overlapping of some of them and suggests that many are probably synonymous; five of these fall within the range of measurements for the smaller form in the writer's material.

Fission and conjugation in the smaller form were studied. Fission followed closely that described by Diller ('28) for a species of *Trichodina* infecting the tadpoles of *R. catesbeiana*, while conjugation was found to closely resemble the process in *Vorticella nebulifera* (Maupas, 1888). Conjugation is anisagamous. A synchronous fragmentation of both macronuclei of each conjugant into small spherical bodies is followed by the establishment of proteoplasmic continuity between conjugants. The contents of the micro-conjugant flow into the macro-conjugant as the micro-conjugant collapses. The ensuing process of post-conjugation is then confined to the single large exconjugant. During this stage the synkaryon gives rise to seven macronuclear anlage each of which by cell division is distributed to daughter cells.

289. THE COLLATERAL CIRCULATION IN THE THIGH OF THE CAT. Homer B. Latimer and Tom G. Orr, Jr., University of Kansas.

The femoral arteries of twelve adult cats (three males and nine females) were ligated just distal to the origin of the profunda femoris. This was done under aseptic conditions and all of the cats survived with only a slight subcutaneous infection in one cat. The cats were sacrificed after from 5 to 12 days. One cat was killed on the third day.

Each cat was watched as it recovered from the ether anesthesia and in no cat was there at any time any visible loss of function in the operated limb, compared with the unoperated. Some of the more docile cats were turned loose in the animal room a day or two following the operation and in very strenuous exercise there was no visible impairment of function in the operated limb.

At autopsy each femoral artery was tested and in every case the ligation had been complete and no fluid could be forced past the double ligation in the artery. There was an apparent enlargement of the obturator and gluteal arteries on the operated side.

290. ON THE USE OF THE PHOTONREFLECTOMETER IN SEROLOGICAL COMPARISONS. Alan Boyden, Rutgers University.

The need of an accurate and rapid method for the titration of precipitating antisera has long been evident. The ring test technique which has given some

results of great value in the comparison of the sera of related species suffers from the handicap that it can fix only one point on the curve of interaction between an antiserum and the antigens with which it is tested. The determination of the volumes of precipitate over the whole range of interaction has proved to be too laborious. The development of the photoreflexometer, "an instrument for the measurement of turbid systems," by Raymond Libby, has filled the need and has given a new impetus to the serological analysis of animal relationship. It is now possible to titrate precipitating antisera rapidly and accurately, with their homologous and heterologous antigens, over the whole range of their interactions. It is also possible to distinguish closely related species and hybrids. The new procedure in serological comparisons of animal relationship consists of (1) the testing of each antiserum with the sera of closely related species or hybrids of known genetic relationship, followed by (2) the testing of such antisera with more distantly related species of less certain genetic relationship. Common mammals have been compared in this way with results in accord with their genetic relationships. It is assumed that antisera which correctly portray genetic relationships where they are known, may be applied with confidence to the study of species of less certain genetic relationship.

291. NATIVE CELLULOSE IN PHALLUSIA NIGRA TUNIC SHOWN BY X-RAY DIFFRACTION MEASUREMENT. Oscar W. Richards, Research Department, Spencer Lens Co., and John Spence, Research Laboratories, Eastman Kodak Co.

The tunic of this ascidian collected at Fort Jefferson, Dry Tortugas, preserved in F.A.A., washed and dried, with and without tension, has been examined by x-ray diffraction. The spacings and intensities of the diagrams are sufficient to confirm the presence of native cellulose crystallites. This result follows from a similar identification of native cellulose in the tunic of *Phallusia mamillata* by Herzog and Gommel (Zeit. Physiol. Chem., 1924, 141: 63).

292. SPONTANEOUS OUTGROWTHS IN *DUGESIA TIGRINA* (SYN. *PLANARIA MACULATA*). E. D. Goldsmith, Washington Square College, New York University.

A planarian with two dorsal pigment 'stripes' instead of the one which is typical for *Dugesia tigrina* was observed in a stock culture which had been in the laboratory for 3 years. Further examination with the aid of the binocular disclosed that the animal had two pharynges. The planarian was isolated; 4 weeks later a small structure growing from the dorsal surface of the postpharyngeal region was visible. Within 2 months, two additional outgrowths appeared.

In an attempt to produce a clone from this animal a transverse cut was made directly anterior to the pharynges. At suitable intervals the operation was repeated and at present eight animals, all derived from the original by transverse splitting, are alive.

It should be noted that the transverse cuts were not followed by the usual regeneration of a typical single head at the anterior end of the posterior piece and of a typical single tail at the posterior end of the anterior piece. Instead, multiple heads (in one case with eight vesicles and pigment cups) and multiple tails such as those which arise following the splitting operation (Goldsmith, '39)

developed. Further, a dorsal outgrowth also developed in one of the animals produced by the transverse cuts.

The outgrowths resemble those produced experimentally by a variety of stimuli (Goldsmith, '34). As soon as an adequate stock is available, an attempt will be made to ascertain whether we are dealing with a strain with a very high regenerative potency and whether outgrowths can readily be produced experimentally.

293. OXYGEN AS A FACTOR IN THE POLARITY OF CORYMORPHA. Victor Schechter, College of the City of New York.

During the summer of 1939 at the Scripps Institution of Oceanography, the author investigated the effect of lowering the oxygen content of sea water upon the reconstitution of *Corymorpha* hydranths. It was found that reconstitution was noticeably inhibited at oxygen tensions beginning at approximately 1 cc. below the normal content in sea water. Inasmuch as it is known that an existing *Corymorpha* hydranth exerts an inhibiting effect on hydranth formation by bordering stem regions, oxygen concentration becomes a factor controlling polarity. In nature, where *Corymorpha* lives with the basal region embedded in the mud, a gradient of oxygen concentration may be assumed to control polarity.

294. INTER-SPECIFIC COMPETITION IN EXPERIMENTAL LABORATORY POPULATIONS. Thomas Park, The University of Chicago.

The present investigation is attempting to assay analytically certain aspects of inter-specific competition in laboratory populations. The organisms used are cereal or meal beetles: *Tribolium confusum*, *Gnathoceros cornutus* and *Trogoderma versicolor*. These forms can be cultured together as mixed-species populations. At regular intervals censuses of larval, pupal and imaginal numbers are taken. The environment is maintained as optimal as it is possible to do so. The experimental populations now running are constituted as follows: control I, consisting of one species only; control II, consisting of two species introduced in initially equal densities, and experiment I, consisting of various species' combinations with one form introduced at a numerical advantage over the other. The experiments have been underway for a year and a half but are still far from completion. However, certain tentative conclusions may be advanced: (1) As single species populations *Tribolium*, *Gnathoceros* and *Trogoderma* give evidence of cyclic fluctuations with age; (2) in mixed populations *Tribolium* appears to drive out *Gnathoceros* and *Trogoderma* irrespective of initial densities, and (3) in mixed populations *Trogoderma* and *Gnathoceros* appear to be about evenly matched; their competition results in no decided advantage for one species over the other.

Attention is again called to the fact that these conclusions are preliminary only and are also somewhat simplified. Nevertheless, they are suggestive and indicate that an empirical study of inter-specific competition above the microorganism level can be approached from the analytical and quantitative viewpoint.

295. SPECIATION BY ECOLOGIC SELECTION, AS SUGGESTED BY STUDIES ON THE LIMPETS OF THE GENUS *ACMAEA* ESCHSCHOLTZ. Avery R. Grant, University of California and San Jose State College. (Introduced by S. F. Light.)

Since eleven species of limpets of the genus *Acmaea* live in close proximity on the north coast of California, all eleven often being found within areas a few

hundred feet in extent, it seemed probable that they had resulted from some method of speciation other than geographic. A study of the natural history and taxonomy of these species shows that there is marked ecologic segregation of taxonomically related species (of the same subgenus), but not of species of different subgenera. This leads naturally to the conclusion that ecologic selection of extreme variants suited to ecologic situations other than those characteristic of the parent species could have, and probably has, been responsible for the origin of many of the neighboring, closely related, but ecologically different species of this genus.

Among the ecologic features which differ markedly within small areas in the intertidal zone, the region occupied by most species of this genus, and which are of critical import to limpets, are exposure to desiccation, exposure to wave action, nature of the substrate, abundance of suitable food, protection from predation.

Variation in any one of these factors, among others, may make a given location impossible for the normal individuals of any species, but make it suitable for individuals which show extreme variations tending toward adaptation for the particular condition concerned. From such variants, selection of offspring of the extreme type would naturally ensue because of the peculiarities of the situation, resulting in eventual speciation due to constant selection and ecologic isolation.

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